Farm Vestn 2008
UDK 615 CODEN FMVTA, SLO ISSN 0014-8229

Farmacevtski vestnik

7th Central European Symposium on Pharmaceutical Technology and Biodelivery Systems

September 18-20, 2008, Hotel Mons, Ljubljana, Slovenia

SPECIAL ISSUE · SEPTEMBER 2008 · LETNIK 59

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September 18-20, 2008, Hotel Mons, Ljubljana, Slovenia

Special Issue, September 2008, Volumen 59

7th Central European Symposium
on Pharmaceutical Technology
and Biodelivery Systems
Proceedings from the Symposium

PUBLISHER

SLOVENIAN PHARMACEUTICAL SOCIETY
Dunajska 184 A, SI- 1000 Ljubljana,
SLOVENIA
Phone: 00 386 1 569 26 01 (3)
Fax: 00 386 1 569 26 02
e-mail: info@sfd.si, home page: www.sfd.si

Printing office: Collegium Graphicum.

The Symposium is supported by the
Slovenian Research Agency

Guest Editors Preface

The aim of the 2008 symposium is from the very beginning to focus on new achievements in pharmaceutical technology and especially in the development of biodelivery systems. The participants were asked to contribute presentations under the three groups of topics: drug delivery systems, pharmaceutical technologies and processes and biopharmaceutic evaluation.

Our expectations were fully met by the authors of 5 plenary lectures in the main symposium and 8 in the satellite symposium, 25 keynote lectures and 154 poster presentations. Drug delivery systems are presented in the broadest meaning of the word, from functionality-related characteristics of the components (drugs and excipients) to their implementation into new dosage forms and/or new production technologies, then to characterize them physically, technologically, chemically and finaly to prove their efficacy and safety under physiological conditions. More precisely, within the scope of the symposium internationally established researchers provide their view on contemporary topics like designing of micro- and nanosized delivery systems and discussing the possibilities and advantages of using stem cells as new drugs. Moreover, new approaches in drug delivery systems design and evaluation in terms of developing in vitro, ex vivo, in vivo and in silico experimental models for the study of mechanisms and kinetics of LADME processes, are open for critical discussion. For the sake of simplicity, presentations demonstrate the diversity of modes how to convert a drug, either small sized pharmaceuticals or macromolecular biopharmaceuticals, into a medicine.

Over 250 symposium participants from 21 countries give this symposium a really international character and represent a guarantee for creative scientific interactions and wide dissemination of new ideas. In this connection we owe a great debt of thanks to all cosponsoring and supporting organizations and societies for their assistance in making this symposium more visible in international scientific community.

Prof. Dr. Aleš Mrhar, President of the Symposium
Assist. Prof. Dr. Saša Baumgartner, General Secretary of the Symposium

SUBJECT INDEX

FINAL PROGRAM

INVITED PLENARY LECTURES

SATELLITE LECTURES

ORAL PRESENTATIONS

POSTER PRESENTATIONS

Milijoni kapljic. En biser.

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FINAL PROGRAM
7th Central European Symposium on Pharmaceutical Technology and Biodelivery Systems

Thursday, September 18th 2008

Satellite symposium: **Challenges and opportunities in multiparticulate drug delivery**
(20th Symposium of the Section of Pharmaceutical Technologists at Slovenian Pharmaceutical Society)

12.00 – 19.00	REGISTRATION
13.00 – 13.15	OPENING CEREMONY
13.15 – 14.00	C. M. Lehr *Saarland University, Biopharmaceutics and Pharm.Technology, Saarbrücken, Germany* **NANOMEDICINE FOR DRUG DELIVERY ACROSS EPITHELIAL BARRIERS**
14.00 – 14.30	G. Borchard *University of Geneva, School of Pharmaceutical Sciences, Geneva, Switzerland* **IS THERE A NEED FOR NANOTECHNOLOGY IN IMMUNOTHERAPY?**
14.30 – 15.00	P. Ahlin Grabnar (1), J. Krist (1) 1, J. Kerč(2) *1 University of Ljubljana, Faculty of Pharmacy, 2 Lek Pharmaceuticals d.d., Ljubljana, Slovenia* **THE MANUFACTURING TECHNIQUES OF VARIOUS DRUG LOADED POLYMERIC NANOPARTICLES**
15.30 – 16.00	K. Mäder *Martin-Luther-University of Halle-Wittenberg, Pharm. Technologie u. Biopharmazie, Halle/Saale,Germany* **MICROPARTICLES AND MICROCAPSULES – STILL HOT OR TOO BIG IN THE NANO-ERA?**
16.00 – 16.30	M. Homar (1), M. Gašperlin (2), J. Kerč (1) *1 Lek Pharmaceuticals d.d., 2 University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia* **PRACTICAL EXAMPLES AND APPLICATIONS OF MICROPARTICLES**
16.30 – 17.00	P. Kleinebudde *Heinrich-Heine-University, Institute of Pharmaceutics and Biopharmaceutics, Duesseldorf, Germany* **PELLETS – A UNIVERSAL TOOL FOR ORAL DRUG DELIVERY**
17.00 – 17.30	J. Kos *University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia* **ANALYTICS OF BIOPHARMACEUTICS IN MULTIPARTICULATE SYSTEMS**
17.30 – 18.00	P. Maincent *University of Nancy, Laboratoire de Pharmacie Galénique, Nancy, France* **CRITICAL REGULATORY ISSUES FOR NEW DRUG DELIVERY SYSTEMS**
18.00 – 18.15	CLOSING REMARKS
19.00	WELCOME RECEPTION

Friday, September 19th 2008

8.00 – 19.00	REGISTRATION
8.45 – 9.00	OPENING CEREMONY
9.00 – 9.45	P. Couvreur *Université de Paris-Sud, Physico-chimie, Pharmacotechnie et Biopharmacie, Chatenay-Malabry, France* **NANOTECHNOLOGY FOR THE CONCEPTION OF NEW MEDICINES: FROM WHERE ARE WE COMING AND WHERE ARE WE GOING?**
9.45 – 10.05	K. Wernig (1), M. Griesbacher (1), S. Hensler (3), F. Hajos (3), F. Andreae (2), W. Mosgoeller (3), J. Wagner (4), A. Zimmer (1) *1 Department of Pharmaceutical Technology, University of Graz, Austria; 2 piCHEM Research and Development, Graz, Austria; 3 Institute of Cancer Research, Medical University of Vienna, Vienna, Austria; 4 Institute for Electron Microscopy and Fine Structure Research, Graz University of Technology, Austria* **PROTAMINE-OLIGONUCLEOTIDE NANOPARTICLES AS DRUG DELIVERY SYSTEM FOR VASOACTIVE INTESTINAL PEPTIDE**
10.05 – 10.25	P. Kocbek, K. Teskač, S. Gobec, J. Krist *University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia*

FORMULATION AND EVALUATION OF NEW DRUG CANDIDATES IN NANODELIVERY SYSTEM

10.25 – 10.45 L. Bildstein, H. Hillaireau, C. Dubernet, P. Couvreur
Université Paris-Sud XI, Faculté de Pharmacie, Châtenay-Malabry Cedex, France
FREEZE-DRYING OF SQUALENOYLATED NUCLEOSIDE ANALOGUE NANOPARTICLES

10.45 – 11.15 BREAK

11.15 – 12.00 P. Simard, J.C. Leroux
University of Montreal, Faculty of Pharmacy, Canada Research Chair in Drug Delivery, Montreal, Canada
PH-SENSITIVE LIPID/POLYMER COMPLEXES WITH APPLICATIONS IN DRUG DELIVERY

12.00 – 12.20 J. Hombach, A Bernkop-Schnürch
University of Innsbruck, Institute of Pharmacy, Innsbruck, Austria
CHITOSAN PARTICLES TO OVERCOME THE BLOOD BRAIN BARRIER

12.20 – 12.40 I. Ozcan (1), F. Segura-Sánchez (2), K. Bouchemal (2), O. Ozer (1), T. Guneri (1), G. Ponchel (2)
1 Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Izmir, Turkey; 2 Paris-South University, Faculty of Pharmacy, Physicochimie Pharmacotechnie Biopharmacie, Chatenay-Malabry, France
DESIGN OF NANOPARTICLES COMPOSED OF POLY (BENZYL GLUTAMATE) DERIVATIVES FOR TARGETED DRUG DELIVERY TO BONE

12.40 – 13.00 R. Cortesi (1), E. Esposito (1), M. Marastoni (1), E. Menegatti (1), C. Nastruzzi (2)
1 Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Ferrara, Italy; 2 Dipartimento di Chimica e Tecnologia del Farmaco, Università di Perugia, Perugia, Italy
PEPTIDE-BASED CATIONIC LIPOSOMES AND MICELLES AS A NEW TOOL FOR NONVIRAL GENE DELIVERY

13.00 – 15.00 LUNCH BREAK / POSTER SESSION

15.00 – 15.45 P. Kleinebudde
Heinrich-Heine-University, Institute of Pharmaceutics and Biopharmaceutics, Duesseldorf, Germany
SOLID LIPID EXTRUSION

15.45 – 16.05 Rok Šibanc (1), Rok Dreu (1), Matjaž Perpar (2), Iztok Žun (2), Stane Srčič (1)
1 University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia; 2 University of Ljubljana, Faculty of Mechanical Engineering, Ljubljana, Slovenia
CFD SIMULATION OF TWO-PHASE FLOW IN WURSTER CHAMBER AND ITS EXPERIMENTAL VALIDATION

16.05 – 16.25 N. Pöllinger (1), A. Prasch and B. Schlütermann
1 Glatt GmbH, Binzen, Germany; 2 ADD Technologies, Reinach, Switzerland
INNOVATIVE FLUID BED PELLETISING TECHNOLOGIES FOR MATRIX PELLETS AND MICROPELLETS

16.25 – 16.45 D. Zupančič Božič (1), T. Vrbanec(1), F. Vrečer (1,2)
1 KRKA d.d., Novo mesto, Novo mesto, Slovenia; 2 University of Ljubljana, Faculty of pharmacy, Ljubljana, Slovenia
NEAR INFRARED SPECTROSCOPY (NIR) AS A METHOD FOR TABLET CRUSHING STRENGTH DETERMINATION

16.45 – 17.05 BREAK

17.05 – 17.25 P. Kása Jr., I. Jójárt, K. Pintye-Hódi
University of Szeged, Department of Pharmaceutical Technology, Szeged, Hungary
PREPARATION OF ASCORBIC ACID CONTAINING CHEWING GUM TABLET BY DIRECT COMPRESSION

17.25 – 17.45 Y.Y. Chen (1), L.F. Gladden (1), L. Hughes (2), M. D. Mantle (1)
1 Department of Chemical Engineering, University of Cambridge, United Kingdom; 2 AstraZeneca, Macclesfield Site, United Kingdom
QUANTIFYING WATER UPTAKE INTO CONTROLLED DRUG DELIVERY POLYMERS USING ULTRA-FAST MRI METHOD

17.45 – 18.05 S. Agatonovic-Kustrin, B.D. Glass, M. Mangan, J Smithson
School of Pharmacy and Molecular Sciences, JCU, Qld 4811
DETERMINING THE CRYSTAL PURITY OF MEBENDAZOLE RAW MATERIAL AND STABILITY IN SUSPENSION BY DRA-UV-SPECTROSCOPY, ATR-FTIR SPECTROSCOPY AND ANN SPECTRAL MODELLING

18.05 – 18.25 G. Marosi, A. Szabó, B. Vajna, G. Patyi, P. Anna
Budapest University of Technology and Economics, Centre of Pharmaceutical and Safety Materials at Dept. of Organic and Chemical Technology, Budapest
TWIN SCREW EXTRUSION, COMPRESSION AND COATING PROCESSES ANALYZED BY TSC, MICRO-RAMAN AND AFM TECHNIQUES

20.00 BANQUET

Saturday, September 20th 2008

9.00 – 9.45 K.H. Krause
Centre Medical Universitaire, Depts. of Pathology, Immunology, and Clinical Pathology, Geneva, Switzerland
EMBRYONIC STEM CELLS AND CELL TERAPY: CELLS AS NEW DRUGS

9.45 – 10.05 M.Cetin (1), I. Vural (2), E. T. Demir (1), A. Atila, Y.Capan (2), Y. Kad oglu (3)
1 Department of Pharmaceutical Technology, Faculty of Pharmacy, Ataturk University, Erzurum; 2 Department of Pharmaceutical Technology, Faculty of Pharmacy, Hacettepe University, Ankara; 3 Department of Analytical Chemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey
PREPARATION AND IN VITRO CHARACTERIZATION OF ANTICANCER DRUG LOADED-IMPLANTABLE PLGA MICROPARTICLES

10.05 – 10.25 U. Bock, E. Haltner-Ukomadu
Across Barriers GmbH, Saarbruecken, Germany
ESTABLISHMENT AND VALIDATION OF AN EX VIVO HUMAN CERVICAL TISSUE MODEL FOR LOCAL DELIVERY STUDIES

10.25 – 10.45 J. Trontelj, M. Bogataj, S. Žakelj, A. Mrhar
University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia
RALOXIFENE PRE-SYSTEMIC METABOLIC CLEARANCE IN THE INTESTINAL WALL AND IN THE LIVER

10.45 – 11.15 BREAK

11.15 – 12.00 J. Kristl
University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia
CURRNET STATUS AND FUTURE OF DERMATICS AND COSMETICS

12.00 – 12.20 E. Csányi (1) and A. Fehér (2) 1 University of Szeged, Faculty of Pharmacy, Department of *Pharmaceutical Technology, Szeged, Hungary; 2 EGIS Pharmaceuticals PLC, Budapest, Hungary*
IN-SITU FORMING LYOTROPIC LIQUID CRYSTALS AS DRUG DELIVERY SYSTEMS

12.20 – 12.40 R. Ambrus (1), P. Kocbek (2), R. Šibanc (2), J. Kristl (2), P. Szabó-Révész (1) 1 University of Szeged, *Department of Pharmaceutical Technology, Szeged, Hungary; 2 University of Ljubljana, Faculty of Pharmacy, Aškerceva 7, 1000 Ljubljana, Slovenia*
NANOSUSPENSION FORMULATION TO IMPROVE THE DISSOLUTION RATE OF MELOXICAM

12.40 – 13.00 T. Gülsün, N. Gürsoy, L. Öner
Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Turkey
CHARACTERIZATION OF NANOCRYSTAL FORMULATIONS WHICH CONTAIN POORLY WATER SOLUBLE DRUG EZETIMIBE

13.00 – 15.00 LUNCH BREAK / POSTER SESSION

15.00 – 15.20 M. Bogataj, A. Bevc, I. Locatelli, A. Mrhar
University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia
DEVELOPMENT OF RELEASE TESTING PROCEDURE FOR DICLOFENAC SODIUM SLOW-RELEASE TABLETS

15.20 – 15.40 B. Ivic (1), S. Ibric (2), G.Betz (3), B. Vranić (2), Z. Djuric (2)
1 R&D Institute, Galenika a.d.,Belgrade, Serbia; 2 Institute for Pharmaceutical Technology, Faculty of Pharmacy, Belgrade, Serbia; 3 Industrial Pharmacy Group, Department of Pharmaceutical Sciences, University of Basel, Switzerland
OPTIMIZATION OF DRUG RELEASE FROM COMPRESSED MULTIPARTICULATE UNITS USING GENERALIZED REGRESSION NEURAL NETWORK

15.40 – 16.00 P. Szabó-Révész, A. Szûts
University of Szeged, Department of Pharmaceutical Technology, Szeged, Hungary
STUDY OF THE EFFECTS OF DRUGS ON THE STRUCTURES OF SUCROSE ESTERS AND THE EFFECTS OF SOLID-STATE INTERACTIONS ON DRUG RELEASE

16.00 – 16.20 Z. Vitková (1), A. Vitko (2), M. Šubová (1), L. Jurišica (2)
1 Comenius University, Faculty of Pharmacy, Bratislava, Slovakia; 2 Slovak University of Technology, Faculty of Electrical Engineering and Information Technology, Bratislava, Slovakia
SYSTEM APPROACH TO FINDING SURROGATES FOR IN -VIVO STUDIES OF SPECIAL DOSAGE FORMS

16.20 – 16.40 R. Starič, I. Legen, P. Jurkovič, J. Kerč
Lek Pharmaceuticals d.d., Sandoz Development Center Slovenia, Ljubljana, Slovenia
DEVELOPMENT AND EVALUATION OF A NEW SOLID STATE MICROEMULSION BASED DRUG DELIVERY SYSTEM

16.40 – 17.00 W. Snor (1), E. Liedl (1), P. Weiss-Greiler (1), P. Wolschann (1), H. Viernstein (2)
1 University of Vienna, Institute of Theoretical Chemistry, Vienna, Austria; 2 University of Vienna, Department of Pharmaceutical Technology and Biopharmaceutics, Vienna, Austria
DENSITY FUNCTIONAL THEORY CALCULATIONS ON MELOXICAM-β-CYCLODEXTRIN INCLUSION COMPLEXES

17.00 – 17.30 CLOSING REMARKS

Friday, September 19th 2008

13.00 - 15.00 POSTER SESSION – 1 – 75

Saturday, September 20th 2008

13.00 - 15.00 POSTER SESSION – 76 – 157

Organizing Societies:

University of Ljubljana
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IP001

Nanotechnology for the conception of new medicines: from where are we coming and where are we going?

P. Couvreur

Université Paris-Sud, UMR CNRS 8612, Chatenay-Malabry

Polymer (1) and lipid-based nanotechnologies (2) were developed and are now proposed as an alternative to classical formulations for drug administration, delivery and targeting (3). A better fundamental knowledge of the in vivo interaction of nanoparticles with biological fluids has led, in the past, to the tailoring of systems efficient, after intravenous administration, in targeting the macrophages of the reticuloendothelial system (the Kupffer cells of the liver or the macrophages of the spleen and of the bone marrow). This specific tissue and cells distribution is explainable by the opsonization processes which occur at the surface of these carriers. Therapeutic applications of these systems include the treatment of cancer liver diseases (4, 5). On the contrary, avoiding the recognition by the liver and the spleen is also possible by developing long circulating polymeric colloidal carriers ("stealth" systems) able to avoid the opsonization process and the recognition by the macrophages. The design of such carriers is based on the physicochemical concept of the “steric repulsion”: by grafting polyethyleneglycol chains at the surface of nanoparticles, the adsorption of seric proteins may be dramatically reduced due to steric hindrance. Such an approach allows maintaining the drug carrier for a longer time into the circulation and the resulting extravasation towards non reticuloendothelial-located cancers may become possible (6). Now, new applications and exciting perspectives are proposed for the delivery of drugs to previously non accessible diseased sanctuaries, like the brain (treatment of glioma and autoimmune diseases of the brain) or the ocular tissues (treatment of the autoimmune uveitis). In these specific diseases, the production of cytokines makes the vascular endothelium dramatically permeable to certain types of nanoparticles, which open options for new medicines (7, 8). It is not questionable if new research programs are needed to better understand the role of cytokines on the endothelium permeability as well as the influence of the molecular coverage of the nanoparticles on their extravasation ability. From those researches, more rationale strategies for the targeting of inflamed tissues should emerge.

New generation of polymer based nanotechnologies allow also the efficient targeting of cells expressing certain receptors, markers and/or antigenic determinants. As an illustration of this approach, folate decorated nanoparticles were found to recognize in a highly specific manner cancer cells with hyper expression of the folic acid binding protein (9).

During these last decades and as discussed above, all the efforts done have concentrated to design efficient nanotechnologies for the targeting of chemical entities towards the diseased areas; the 21st century could turn into the development of nanotechnologies for the targeted delivery of physical treatments (ie.of tumors) (9, 10). This approach will need to perform original chemistry with metallic-core based nanoparticles but also to improve our knowledge on the interaction of electromagnetic waves with living tissues.

Crucial is also the need to find intelligent nanomedicines to overcome resistances to chemotherapies both in the field of cancer diseases and viral infections. In this view, the regulation of the cellular drug influx/efflux, the protection against enzymatic intracellular metabolization as well as the control of the intracellular drug trafficking are a key factors of the success. This will be illustrated by a new approach based on the *squalenization* technology which allows the conception of efficient nanomedicines for the treatment of resistant leukemia (11, 12) and HIV infections (13).

Finally, the use of nanotechnologies for the intracellular delivery of short fragments of nucleic acids (oligonucleotides and siRNA) is certainly a field of investigation in which research efforts should let to breakthrough discoveries incl. the possibility to inhibit the expression of junction oncogenes and to reverse the cancer phenotype as it was recently shown with the ret/PTC1 oncogene in the papillary carcinoma of the thyroïd (Tchernobyl disease)(14).

This lecture is an overview of what could be the research on nanotechnologies in the near future. The message is that to be efficient, it should have a strong Pharmaceutical flavour at the frontier of the Physics, the Chemistry and the Biology.

References

1. Kim et al., *CMLS*, 64, 356-364 (2007)
2. Angelov et al., *J Amer. Chem. Soc.*, 128, 5813-5817 (2006)
3. Brigger et al, *ADDR*, **54**, 631-651 (2002)
4. Colin de Verdière et al., *Brit. J. Cancer*, **76**, 198-205 (1997)
5. Barraud et al., *J. Hepathology*, **42**, 736-743 (2005)
6. Brigger et al., *JPET*, **303**, 928-936 (2002)
7. Calvo et al., *Europ. J. Neurosci.*, **15**, 1317-1326 (2002)
8. De Kozac et al., *Europ. J. Immunol*, **34**, 3702-3712 (2004)
9. Sonvico et al., *Bioconj Chem*, **16**, 1181-1188 (2005)
10. Dimarco et al., *J Am. Chem. Soc.*, **128**, 10054-10059 (2006)
11. Couvreur et al., *Small*, in press (2007)
12. Reddy et al., *J Control Release*. **124**, 20-27 (2007)
13. Couvreur et al, *NanoLetters*, **6**, 2544-2548 (2006)
14. deMartimprey et al., *NAR*, in press (2007)

IP002

pH-sensitive lipid/polymer complexes with applications in drug delivery

Pierre Simard, Jean-Christophe Leroux

Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University of Montreal, P.C. 6128 Downtown Station, Montreal (Qc), Canada, H3C 3J7

INTRODUCTION

A promising strategy in carrier-based drug delivery for cancer therapy consist in the combination of site-specific delivery with triggered drug release. While the presence of a monoclonal antibody (mAb) on the surface may specifically increase the targeting of malignant cells and favour the endocytosis of the carrier, the use of trigger mechanisms may be useful to increase the intracellular drug bioavailability in these cells (**1**). Indeed, once the encapsulated material is endocytosed, it may eventually be delivered to the lysosomal compartment, where it can be easily destroyed or inactivated by various enzymes (*i.e.* hydrolases, peptidases). This is the case, for example, with the cytosine arabinoside (ara-C) commonly used in the treatment of acute myeloid leukemia (AML). For such biologically unstable molecule, methods to facilitate the release of the entrapped cargo in the cytosol are highly desired.

Here, we propose a polymer-based pH-sensitive immunoliposomes (ILs) system for ara-C that is designed to release its content inside the endosomal compartment through polymer structural change following receptor-mediated internalization. The ILs were rendered pH-sensitive by including a copolymer of *N*-isopropylacrylamide (NIPAM) and methacrylic acid (MAA) which undergoes a hydrophilic-hydrophobic transition at acidic pH (**2**). The latter destabilizes the liposome bilayer and favour the release of the drug before it is degraded in the lysosomes. Moreover, these carriers were targeted with mAbs directed against the CD33 receptor, a cell surface marker expressed on more than 80% of leukemia isolates of patient suffering from acute myeloid leukemia (AML) (**3**). These ara-C loaded ILs-CD33 could thus serve as an effective formulation against AML. In this work, the specificity, pH-responsiveness and *in vitro* efficacy of these pH-sensitive ILs-CD33 was investigated.

EXPERIMENTAL METHODS

Preparation of the copolymer and pH-sensitive ILs

The terminally-alkylated polymer (M_w=11,000, PI= 2.1) was synthesized by radical polymerization of NIPAM and MAA (95:5 molar ratio) in the presence of a dioctadecylamide-based (DODA) initiator. Liposomes (egg phosphatidylcholine / cholesterol / poly(ethylene glycol) $(PEG)_{2000}$-distearoyl phosphatidylethanolamine (DSPE) /maleimide-PEG-DSPE, 3.2:0.17:0.09 molar ratio) with size of ~160 nm were prepared by hydrating the lipid film with a solution of either 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, 35 mM)/*p*-xylene-bis-pyridinium bromide (50 mM), ara-C (200 mM) or calcein (120 mM), followed by extrusion through polycarbonate filters. The DODA-P(NIPAM-*co*-MAA) was added to the lipid mixture at a ratio of 0.12-0.3 (*w/w*) prior to the hydration step. The anti-CD33 mAb (clone P67.6) was modified with 3-(2-pyridyldithio) propionyl hydrazide at the carbohydrate sites and conjugated to maleimide-PEG-DSPE anchored onto the surface of preformed liposomes.

In vitro release

The release of both the quenched fluorescent probe HPTS and the anticancer agent ara-C was done by first incubating the vesicles in 50% (*v/v*) human plasma for 1 h at 37 °C. The excess of plasma components was removed by gel permeation chromatography and the release of HPTS from the vesicles was monitored over 30 min at 37 °C at different pHs (5.0, 5.8, and 7.4). The percentage of ara-C released from ILs was determined after a 30 min incubation at pH 5.0 and 7.4.

Internalization and cellular localization assays

Leukemic HL60, KG-1, THP-1 (CD33+) and lung carcinoma A549 (CD33-) cells were incubated 2 h with ILs labelled with the hydrophobic probe BODIPY FL C12. Fluorescence-associated cells were sorted by flow cytometry. The intracellular release of calcein from the ILs was observed by confocal fluorescence microscopy.

Antiproliferative assay

Cellular cycles were synchronized to the S-phase through incubation with 2 mM of thymidine before the antiproliferative assay. Ara-C-loaded ILs were added to HL60 cells, and incubated for 2 h at 37°C. Cells were then rinsed with phosphate buffered saline, and supplied with fresh culture medium. Cell proliferation was determined by the reduction of methylthiazolyldiphenyl-tetrazolium bromide.

RESULTS AND DISCUSSION

In this study, pH-sensitive ILs were obtained by including the terminally-alkylated copolymer DODA-P(NIPAM-*co*-MAA) in the preparation of PEGylated liposomes and coupling the anti-CD33 mAb through a thioether bond. *In vitro* release of encapsulated HPTS, calcein and ara-C revealed that pH-sensitivity of the vector was retained in the presence of the antibody upon incubation in human plasma. Protein adsorption did not affect the stability bilayers of this polymer-based system.

Flow cytometry and confocal microscopy analysis demonstrated that the pH-sensitive ILs were efficiently internalized by various CD33+ leukemic cell lines (HL60, KG1, THP-1) while limited interaction was found for liposomes decorated with an isotype-matched control antibody (MOPC) (Fig. 1). Competitive binding assays on HL60 cells confirmed the specificity of the interaction. Following internalization, pH-sensitive ILs efficiently released their payload inside the cells, as evidenced by the appearance of diffuse intracellular unquenched calcein fluorescence acquired by confocal microscopy. On the other hand, mainly punctuate flu-

orescence was observed with control non pH-sensitive ILs and with pH-sensitive ILs in the presence of the endosomal acidification inhibitor, bafilomycin A1, indicating that intracellular release of calcein was dependent on pH-sensitivity. Finally, the highest antiproliferative effect was obtained for pH-sensitive ILs-CD33, against HL60 cells, confirming the role of the DODA-P(NIPAM-*co*-MAA) copolymer in promoting the escape of ara-C before its degradation into lysosomes.

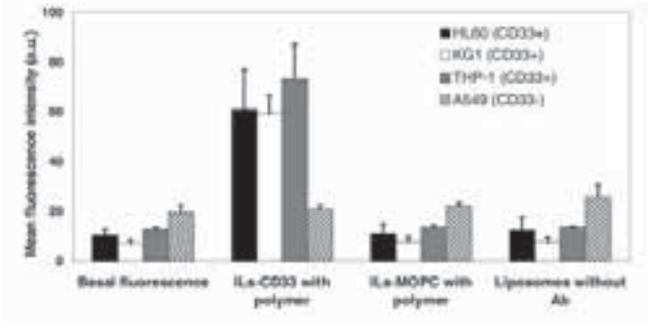

Figure 1: Flow cytometry analysis of uptake of pH-sensitive ILs by different cell lines (Mean ± SD, n=3).

CONCLUSION

This study describes a targeted pH-sensitive liposomal formulation specific toward leukemia cells expressing the CD33 receptor. DODA-P(NIPAM-*co*-MAA) was shown to destabilize the bilayer membrane at endosomal pH to provide significant benefits in the intracellular release of labile encapsulated agents.

ACKNOWLEDGMENTS

This work was financially supported by the Canadian Institutes of Health Research and the Canada Research Chair Program. P. Simard acknowledges a scholarship from the Fonds de la Recherche en Santé du Québec.

References

1. D. C. Drummond *et al.*, Prog. Lipid. Res. *39*; 409 (2000).
2. E. Roux *et al.*, Biomacromolecules *4*; 240 (2003).
3. D.A. Scheinberg *et al.*, Leukemia *3*; 440 (1989).

IP003

Solid Lipid Extrusion

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SOLID LIPID EXTRUSION

Extrusion is the process where a plastified mass is forced through a die of defined geometry. Two dimensions of the resulting extrudate are defined, only the length can vary. Wet extusion and hot melt extrusion are two well established processes. In wet extrusion the mass is plastified by adding an appropriate amount of a liquid, in most cases water. In hot melt extrusion the plastification is induced by raising the temperature, which leads to the melting of a suitable binder. The molten binder acts like the liquid in wet extrusion.

In solid lipid extrusion a formulation containing powdered lipds is forced through the dies at a temperature below the melting temperature of the lipid. Usually, the temperature is approximately 5K to 15K below the melting temperature of the lipid. Depending on the lipid extrusion can be performed at room temperature and called cold extrusion. The thermomechanial treatment during extrusion results in the required plasticisation of the mass. Although solid lipid extrusion is known since long time [1] the interest in this process raised recently.

Solid lipid extrudates can be produced in a certain temperature range. Determination of the solid content of the lipd shows that only a small fraction of the lipid is molten at the extrusion temperature [2]. The main part remains as a solid.

The extrudates can be cutted into extrudates of a certain length, milled to smaler particles or spheronised into pellets.

SELECTION OF LIPIDS

Different lipids are commercialy available in powdered form. These lipids vary in their composition and their melting ranges. All tested powdered lipids could be successfully used for solid lipid extrusion, if the temperature is adjusted appropriately.

The lipds can vary with respect to the homogeneity of their composition [3]. Some lipids are mixures of mono-, di- and tri-glycerides containing fatty acids of different chain lengths. One example is glyceryl-palmito-stearate (GPS). Other lipids are mainly composed of tri-glycerides of only one chain length. One example for this group is glyceryl-tri-myristate (GTM). GPS and GTM were compared with respect to their suitabiity for solid lipid extrusion. Both have similar melting ranges. Since the melting range of GPS is broader the solid lipid extrusion process is more robust and easier to control. Using GTM even small temperature changes can affect the extrudate properties.

The physical stability of GTM extrudates is good. Since the extrudates contain the stable ß-modification from the beginning changes cannot be observed. This is reflected in the dissolution profile which is also stable after storage. In contrast extrudates with GPS exhibit significant changes in the solid state properties during storage. Also the dissolution profile is not stable during storage. Thus, the use of more homogeneous lipids is recommended for solid lipid extrusion.

Comparing pure tri-glycerides of different chain lengths it could be shown that physically stable extrudates resulted from glyceryl-tri-laurate and glyceryl-tri-palmitate [4]. In case of glyceryl-tri-stearate the solid state depended on the extrusion temperature. When using a temperature of 65°C the extrudates only contained the stable ß-modification of the lipd. However, an extrusion temperature of 55°C led to the formation of a small fraction of the a-modification. This leads to the recomendation to perform extrusion of pure tri-glycerides between the melting temperatures of the α- and the β-modification.

PHARMACEUTICAL APPLICATIONS

Solid lipid extrusion can be used to design multiple unit controlled release formulations. The lipids act as a matrix resulting in a Higuchi release kinetic. Depending on the selected lipid the release rate can be adjusted in a wide range. Furthermore, the release rate depends on the loading with drug. Increasing fraction of drug up to 80% results in a faster dissolution. Solid lipid extrudates can be used for taste masking purposes.

After milling the extrudates show a burst effect. It could be shown that dissolution from the outer cylinder surface of the extrudate is slower than from a broken surface. This is due to a different distribution of lipids and drug at the surface of an extrudate. This knowledge makes the use of small diameters during solid lipid extrusion attractive [5]. After milling to a certain particle size fraction extrudates with small diameters result in a slower dissolution. It was possible to produce solid lipid extrudates with diameters down to 0.3mm.

It is possible to spheronise solid lipid extrudates [6]. A prerequisite is the possibility to heat the spheroniser, e.g. by adjusting the temperature of the spheronsier wall. The spheronisation requires a good control of the process variables, especially the temperature of the product. Cold extrudates are given into the spheroniser. In the begining of the process the brittle extrudates brake into short cyliders. Depending on the spheronisaton process the temperature of the product raises due to the heat transfer from the spheroniser wall and also due to the friction during the movement. The temperature rise leads to a plasticisation of the product, which enables the spheronisation. Round pellets of narrow particle size distribution are obtained.

References

1. Schmidt PC, Prochazka J. Über die Herstellung von Retardgranulaten durch Granulatformung. Pharm Ind 38: 921926 (1976)
2. Reitz C, Kleinebudde P. Solid lipid extrusion of sustained release dosage forms. Eur. J. Pharm. Biopharm. 67: 440-448 (2007).
3. Reitz C, Kleinebudde P. Influence of thermal and thermo-mechanical treatment: Comparison of two lipids with respect to their suitability for solid lipid extrusion. J. Thermal Anal. Calorim. 89: 669-673 (2007).
4. Windbergs M, Strachan CJ, Kleinebudde P. Understanding the solid-state behaviour of solid lipid extrudates and its influence on dissolution. Eur. J. Pharm. Biopharm. (accepted)
5. Michalk A, Hamann H-J, Kanikanti R, Kleinebudde P. Controlled release of active as a consequence of the die diameter in solid lipid extrusion. J. Contr. Rel. (submitted)
6. Reitz C, Kleinebudde P. Spheronization of solid lipid extrudates. Powder Technol. doi:10.1016/j.powtec.2008.04.009

IP004

Embryonic stem cells and cell therapy: cells as new drugs

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EMBRYONIC STEM CELLS

Embryonic stem (ES) cells are pluripotent stem cells derived from early embryos at the blastocyst stage. ES cells can differentiate into virtually any cell type. This property of ES cells is of interest for several reasons: i) studies on basic mechanisms of human cell differentiation; ii) use of ES-cell derived human cells and tissues for in vitro studies (drug developmemt, toxicology, infection models etc.) iii) use of ES-cell derived cells for therapy of diseases which are characterized by cell loss. The use of ES cells for therapeutic purposes is particularly relevant for cell types, which cannot easily obtained otherwise. This is particularly true for cells of the central nervous system, as for example dopaminergic neurons.

CELL THERAPY

Cell therapy refers to the concept to use cells for therapeutic purposes. In the case of cell replacement therapy, cells which are lost (usually in a disease process) are replaced by exogenously provided cells. Thus, cell replacement therapy represents a specialized form of transplantation. Parkinson's disease is an interesting paradigm for cell replacement therapy. In Parkinson's disease, there is a localized loss of dopaminergic neurons in the substantia nigra, as well as their axonal extensions towards the striatum. Thus, there is a good theoretical basis for the concept that implantation of dopaminergic neurons at the appropriate anatomical localization should provide a therapy for the disease. There is now abundant evidence that implantation of ES cell-derived dopamingergic neurons leads to marked improvement in rodent models of Parkinson's disease. There is also emerging evidence that such a cell therapy works in primate models. No ES-cell derived neurons have been used for human therapy so far, but there is some experience with implantation of fetal brain-derived neurons in Parkinson's patients. And, although this source of dopaminergic neurons is less than optimal, there are at least some positive results observed.

THE GENEVA PARKINSON'S PROJECT

To advance towards an embryonic stem cell-based therapy of Parkinson's disease, clinicians and researchers from the Geneva University Hospitals and Medical Faculty have teamed up to create the Geneva Parkinson's Project. This is a multidisciplinary team consisting of experts from the following domains: Neurology, Neurosurgery, Neuroimaging, Transplantation Immunology, Infection Control, Gynecology, Cell Biology, Experimental Pathology, and Medical Ethics. The groups are working closely together on the implementation of a cell therapy approach towards Parkinson's disease. The challenges are manyfold, and patient safety and solid scientific evidence are the most important criteria of the team. Yet, an advance towards cell therapy of Parkinson's disease within a time frame of approximately five years seems realistic.

IP005

Current status and future of dermatics and cosmetics

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INTRODUCTION

Topical application of various dosage forms or compositions, such as creams, lotions, ointments or just oils for cosmetic and therapeutic effects in the skin and local tissues has been used for thousands of years. Dermatics and cosmetics generally signify the topical application, thus targeting skin at the site of application, reducing systemic site effects. Transdermal drug delivery, on the other hand, utilises skin as the site for the administration of systemically active drugs. The range of molecules that can achieve appropriate effect following application to the skin is severely limited. This is due to effective barrier properties of the intact skin. Research of the skin structure since the 1960s has generated an understanding of the processes that are involved in percutaneous penetration, and since 1980s the progress in molecular biology with important implications in skin homeostasis and treatment of skin diseases was shown. This scientific development has profoundly changed numerous concepts of skin physiology and pathophysiology. Additionally, through technical developments in the biotechnology and nanotechnology area, the pharmaceutical and cosmetic industry have gained access to a wide range of new scientific tools and testing abilities which can be successfully applied to new therapeutical approaches and to develop new products including active ingredients as well as dermatic/cosmetic carriers.

CURRENT REGULATORY STATUS

The activity at the level of the epidermis or dermis represents the borderline for dermatic or cosmetic products. In this regard there are three different European Union directives regulating cosmetics, drugs and medical devices, and the three definitions all refer to the biological structures of the skin. In particular, the definitions were compared trying to identify the borderlines and especially the overlapping parts. Moreover, the possibility to better consider and classify the new functional cosmetics - so-called *cosmeceuticals* – was also taken into consideration, since the new trend in skin care products shows a shift towards visible efficacy, leaving aside cosmetic ingredients that only physically modify the skin and turning to those that physiologicaly intervene in the sub-cellular processes of the skin, rendering the classification and regulation of cosmetics/ drugs/medical devices difficult and vague.

NEW VIEW ON SKIN STRUCTURE AND FUNCTION

The skin is the largest organ of our body and acts as a protective barrier with sensory and immunological functions. Being the barrier between the individual and the environment, the skin prevents the entry of microorganisms, blocks radiation, and prevents water loss. Epidermal cells are regularly exposed to marked variations in ambient temperature and humidity, and to physiological stressors such as inflammatory cytokines. Skin solves these activities through the three layer structure (epidermis, dermis and subcutis) and appendixis (sweat and sebaceous glands and hair follicles). Stratum corneum (SC) is part of the epidermis and is the outermost upper layer of the skin. The dermis lies below the epidermis and under it, the subcutaneous tissue. The epidermis which is a self-renewing skin layer must balance the cell proliferation, differentiation and death. Innermost basal layer of viable epidermis adheres to an underlying basement membrane rich in extracellular matrix. This layer contains proliferative keratinocytes that are typified by their expression of genes encoding integrins and growth factor receptors, particularly epidermal growth receptor (EGF), as well as the genes for structural keratins 5 and 14 (K5 and K14). As basal cells move upward, they repress basally expressed genes and switch to the expressing a set of differentiation-associated proteins, including keratins K1 and K10. As keratinocytes continue their trek, they further adjust transcriptional program to cumulate in the production of dead, flattened squames that are sloughed from the skin surface as new cells moving outward replace them.

SC is composed of two domains: protein-rich nonviable cells and intercellular lipid matrix. The lipid composition of the SC differs a lot from the other layers of the epidermis and other tissue. Intercellular layers are composed primarily of three classes of lipids: free sterols, free fatty acids and ceramids derived by enzymatic hydrolysis of lipids in lamellar bodies displaced close to the stratum granulosum - stratum corneum interface. Serious defects in the epidermal keratinocyte lipid transporters ABCA 12 are known to result in a deficient skin lipid barrier. As yet unknown lipid transporters and transport mechanisms other than ABCA 12 may be involved in lipid transport, accumulation, and secretion from human keratinocytes.

Ceramides are attractive molecules for research in molecular biology, as they are pro-vital molecules in multiple biological processes, such as apoptosis, the cascade of processes by which an extracellular signal interact with a receptor on the cell surface etc. It is known now that epidermal homeostasis is under tight transcriptional regulation. For example, using conditional knockout technology researchers have discovered that AP-2 alfa is essential for governing the balance between growth and differentiation in epidermis (AP-2, JCB). Although a role for many other transcription factors that modify skin gene expression have been implicated in epidermal biology, a clear picture of their functional significance has remained elusive. It is known that the magnitude of the increase in epidermal DNA synthesis directly correlates with the degree of barrier disruption.

The structure and function of human skin is largely dependent on its underlying connective tissue (dermis), which contains the blood vessels that feed the skin, nerves, sebaceous glands, and hair follicles. The dermal extracellular matrix is largely composed of type I collagen fibrils that support the skin and elastin fibers that provide flexibility. Time- and sun-exposure dependent alterations of collagen fibrillar content and organization impair the structural integrity of skin connective tissue and to altered appearance of human skin. Molecular mechanisms show that cysteine-rich 61 mediator is a pivotal regulator of collagen homeostasis. The gradual loss of epidermis and dermis – skin atrophy – is cosmetically and clinically important because aging skin is more fragile and dry, heals slower than young skin and is also prone to ulceration. Experimental evidence suggests that defective function of the major cell-surface hyaluronate (HA) receptor CD44, which is associated with impaired HA metabolism underlie the pathogenesis of atrophy. Authors suggest that topical administration of defined size hyaluronate fragments may provide therapeutic option in human skin atrophy.

In many skin disorders and disease morphogenesis has been recognized that significant alterations shown as clinical symptoms tend to be more or less associated with barrier dysfunction (atopic dermatitis, psoriasis, contact dermatitis etc). Many present studies try to clarify abovementioned clinical findings, further elucidating of their mechanisms of pathogenesis through identifying the defects in gene families or mediators. Additionally, today sets of transcription-factors binding motifs are identified that are enriched among ageing-regulated genes and highlight areas for future development of cosmetic products.

SKIN ABSORPTION AND PERMEATION

Ability of drug delivery through human skin is important in modern therapy. Although the routes of penetration of molecules through the SC are still highly researched and debated, there is a consensus that small, uncharged molecules permeate via intercellular lipid routes, whereas highly hydrophilic molecules may follow a transcellular pathway, although the bilayered lipid regions that traverses between keratinocytes remain a rate-limiting barrier. Various strategies have been designed to modulate the skin s permeability, in order to promote transdermal delivery of a wide range of drugs. The strategies include the optimization of drug and vehicle properties, and modification of the SC by chemicals or electrical/external force methods.

VEHICLES FOR TOPICAL APPLICATIONS

In the history the skin care, occlusion and moisturization with artificial materials have been used to improve the skin condition. However, such treatments potentially perturb the homeostasis of the skin. In order to develop sophisticated formulations for topical application, the role of the components that are responsible for the barrier formation should be carefully chosen. With increasing emphasis on functional additives in cosmeticals, compounds obtained by biotechnological methods are gaining a key role in recovering the endogenous skin function by accelerating the skin homeostatic process to increase barrier and promoting repair. Such compounds moved the research away from simple occlusion and are certainly leading a way to future skin care systems. Traditional dermatics - semisolids are classified as ointments, pastes, creams and gels. Advances in biotechnology have given rise to numerous protein and peptide entities with therapeutic potential, which pose problems in stability and in the ability to deliver the drug in a patient friendly manner to specific target tissue. To overcome these problems, colloidal drug delivery systems are an increasingly important field of research that could provide better medicines. These colloidal systems have been collected into four major classes: self-assembled lipid systems (micelles, liposomes, microemulsions, nanoemulsions, SLN), polymer systems (polymeric micelles, polymeric nanoparticles, dendrimers), drug nanoparticle system and pro-colloidal systems (self-emulsified systems and liquid crystalline systems). Their particular attributes are a function of their size, surface area, surface modification or encapsulation and solubilization capacity. The decision of which colloidal system to select for the particular application comes down in terms of satisfying the particular attributes required in the product profile.

CONCLUSION

The purpose of the presentation is to compare and contrast some of the more recent developments in skin molecular biology and different types of vehicles developed for topical application. Further, the attempt is to clarify future trends in the skin care and treatment products development.

EDITORIAL

20th symposium of the Section of pharmaceutical technologists at the Slovenian Pharmaceutical Society and Satellite symposium at the 7th Central European Symposium on Pharmaceutical Technology and Biodelivery Systems

Challenges and opportunities in multiparticulate drug delivery

Since 1989 Section of pharmaceutical technologists at the Slovenian Pharmaceutical Society has been organizing a traditional one day symposia. By joining a traditional 20th symposium of the Section of pharmaceutical technologists with the 7th Central European Symposium on Pharmaceutical Technology and Biodelivery Systems, a Satellite symposium was established this year and both symposia gained even greater importance. For this reason the program of satellite symposium was chosen very carefully to fulfill the needs and expectations of a broad audience from academia and industry.

Research and development of multiparticulate drug delivery systems has been very extensive in the last two decades and has followed several routes depending on the final application. Over 25 percent of recent modified release drug approvals in North America and Europe have used multiparticulate technologies and dosage forms including beads, pellets and minitablets and their growing popularity, is borne out by the fact that FDA approvals of multiparticulate formulations have doubled over the past decade. This growth in the utilization of multiparticulate dosage forms is attributed to their superior clinical performance on oral administration. Although a wide variety of systems have been designed with their own advantages and limitations, the common goal is to rationalize drug delivery to enhance the bioavailability of the drugs towards targeted diseased cells, promoting the required response while minimizing side effects. Multiparticulate drug delivery systems also represent unique opportunities to get challenging molecules, such as nucleic acids, proteins and peptides as well as special very potent low molecular compounds, into clinics.

This symposium brings together a group of internationally recognized experts in the science of multiparticulate technologoies. They will showcase the key scientific concepts that underpin multiparticulate technologies and present case studies detailing how key challenges have been met. This symposium is aimed to elaborate the advantages and drawbacks of the individual multiparticulate delivery systems ranging from the smallest nanotechnology based systems to the most popular millimeter sized systems in the form of pellets.

In nanotechnology area an enormous effort has been invested into the research of penetration of such small particles through biological barriers, represented biological barriers of the gastro-intestinal tract, the skin, the lungs and blood brain barrier (BBB). The first lecture will highlight some of the recent results or data of work in progress in these areas, either concerning the development of new in-vitro models or new drug carriers systems, for which the nanosize has often turned out to be advantageous. Another aspect of particulate drug delivery has been the modification of such delivery system to avoid premature interaction with the immune system, e.g., the reticuloendothelial system (RES), to provide for an increase in retention time in systemic circulation and an altered tissue distribution. Nanotechnology at this stage is exemplified by alteration of the surface of particulate carriers, by coupling polymers (surfactants, polyethyleneglycole) through covalent binding or adsorption.

There are several methods that have been employed to load active molecules into polymeric nanoparticles and selection of one over the other depends on the nature of the polymer and the properties of the drug. They can be classified into two main categories according to whether the formulation requires a polymerization or is achived directly from a macromolecule or preformed polymer. Polymerization-based methods may present some drawbacks and limitations such as inadequate biodegradability properties of the product and the presence of toxic residues. In order to circumvent these limitations nanoparticles can be prepared directly from preformed synthetic or natural polymers. Polymers such as polyglycolic acid (PGA), polylactic acid (PLA), polylactic-glycolic acid (PLGA), poly(ε-caprolactone) (PCL), chitosan, albumin, gelatin and alginate are the most common types used for the production of nanoparticulate carrier systems, although there is a host of other novel polymers that have been developed to improve site-specific delivery, controlled release, and drug-loading capabilities.

Besides analytical tools used for characterisation of “classical” parameters of nano-vehicles, such as size, zeta potential and morphology, protein containing nano-vehicles demand the tools for analysis of protein content, protein structure and biological activity, coating efficacy, protein-protein interactions, cell internalisation, etc. For this purpose the application of surface plasmon resonance (SPR), fluorescent microscopy, flow cytometry and other immunological methods will be discussed.

Microparticles and microcapsules also should not be neglected in the nanoera, since micro sized particles offer similar or even better opportunities for special delivery purposes. A wide variety of methods can be used for the preparation of microparticles. The most common include polymerization of monomers, solvent displacement (either by solvent evaporation or the addition of larger volume of outer phase which is partially miscible with the solvent from inner phase), spray drying, and coacervation. Drug release form microparticles is strongly influenced by the materials used for the preparation of the microparticles. A desired dissolution profile can be achieved by proper selection of material or material mixtures. Two examples of microparticles preparation and characterization are described. First example describes preparation of microparticles with

a commonly used emulsion solvent evaporation method using a variety of polymers commonly employed for microparticles preparation. Second example describes preparation of microcapsules with liquid core prepared by vibrating nozzle method, which is rarely described in the literature.

Pellets are the largest particles which are covered in the Symposium. Pellets as multiparticulate systems are advantageous compared to monolithic solid dosage forms, if they provide some biopharmaceutical functionality. This can be enteric resistance or many different ways of a modified release. In many cases pellets are coated to achieve the desired release profile. Many techniques are available for the production of pellets, however, extrusion/spheronisation will be discussed in more detail.

Last but not least critical regulatory issues for new drug delivery systems will be reviewed in order to understand the reasons for the gap between the scientific literature and the low number of marketed new drug delivery systems. Launching a new drug delivery system on the market is very demanding but not impossible. For patented drug delivery systems, there is still a long road from the concept to the market. The difficulties, from a pharmaceutical point of view, are related to the current guidelines existing in Europe but also in the USA and Japan (just to mention the most important world areas). Basically, any company wanting to market a new drug delivery system will have to deal with guidelines on excipients, pharmaceutical development, manufacturing, control of finished product at release but also stability which may be more critical for these systems. The most important guidelines needed to be well known in case of interest for new drug delivery systems will be discussed. The information and knowledge gained from pharmaceutical development studies and manufacturing experience provide scientific understanding to support the establishment of the design space, specifications, and manufacturing controls.

I wish all the participants a very fruitful Symposium and a pleasant stay in Ljubljana. I hope we meet again at the next Symposium of the Section of pharmaceutical technologists at the Slovenian Pharmaceutical Society next year.

Assoc. Prof. Dr. Janez Kerč
President of the Satellite Symposium

SL001

Nanomedicine for drug delivery across epithelial barriers

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Due to the advances of molecular biotechnology and bioinformatics, there is a strong increase of new candidate drug molecules. However, apart from their affinity to the target receptor, bioavailability and transport to the actual site of action is an important issue when it comes to developing such molecules to actual drug products. This holds in particular for macromolecular biopharmaceuticals, such as peptides, proteins, oligonucleotides or gene vectors, but also for some small organic molecules, which may suffer from insufficient or erratic permeability due to the interference with epithelial efflux systems of metabolism. Therefore, alternative routes of drug administration have to be investigated, and new technologies for their improved delivery have to be developed. This also includes the development of some in-vitro models based on relevant epithelial cells or tissues. These test systems allow not only to screen drug candidates with respect to their permeability, but also to evaluate the performance of some advanced delivery technologies in terms of safety and efficacy before moving into animals or man, and to understand their mechanism of action as well as the identification of critical physiological parameters under controlled conditions.

The focus of our research over the past ten years has been on the biological barriers of the gastro-intestinal tract, the skin and the lungs. This presentation will highlight some of our recent results or data of work in progress in these three areas, either concerning the development of new in-vitro models or new drug carriers systems, for which the nanosize often has turned out to be advantageous.

TARGETING OF NANOPARTICLES TO INFLAMED AREAS OF THE INTESTINAL MUCOSA

Inflammatory bowel diseases, such as Morbus Crohn or Colitis Ulcerosa, are painful for the patient and moreover difficult to treat due to the increased mucus production and the occurrence of diarrhea. Rather unexpectedly, we found that nanoparticles (100 nm Fluoresbrite-Particles) showed a significantly increased residence time and selective accumulation in inflamed areas of the colon compared to non-inflamed colonic tissue and the small intestines in a TNBS-induced colitis model in the rat. This effect was, however, only significant for nanoparticles, but not for larger microparticles. In a subsequent study, we could demonstrate that an anti-inflammatory drug (rolipram), when delivered by nanoparticles made of biodegradable PLGA, led to a prolonged alleviation of colitis syndromes and a reduction of central nervous side effects, compared to the same dose of the drug administered as an aqueous solution [1, 2]. While the mechanism of this preferred accumulation of nanoparticles in the inflamed mucosal tissue is still unclear, it clearly indicates an interesting advantage of nanocarriers for this particular application. We are currently in the process to investigate whether thes same effect can also be observed in colitis patients by

confocal laser endoscopy, and to develop an in-vitro model for studying the interaction of nanoparticles with the intestinal epithelium in state of inflammation.

NANOPARTICLES FOR IMPROVED DRUG DELIVERY TO THE SKIN

In order to investigate this new working hypothesis, we encapsulated the non-steroidal anti-inflammatory drug flufenamic acid (FFA) in nanoparticles of PLGA, dispersed it in an HPMC gel formulation and applied it to human skin in vitro. To our surprise, transport of FFA into deeper skin layers was significantly enhanced for such nanoparticle formulation compared to the free drug dissolved in the same hydrogel vehicle [3]. While nanoparticles have been reported to penetrate along hair shafts and to thus accumulate in hair follicles [4], such penetration could not be observed for PLGA nanoparticles in our experiments. Instead, visualization by multiphoton microscopy showed that most of the particles where seen in the intercellular clefts between the keratinocytes [5]. Therofore, the observed enhancement of epidermal penetration must have another reason. As we could demonstrate, during the hydrolysis of PLGA nanoparticles a acidic microclimate is formed, most likely leading to a reduced dissociation and higher lipophilicity/better penetration of flufenamic acid. In contrast, this could not be observed for particles made of traganth/gummi arabicum. This result is of particular interest, as it points out that their small size only may be not sufficient to explain all the unusual effects that one may observe with nanoparticles. Instead, the chemical composition and materials the nanoparticles are made of are at least evenly important.

NANOPARTICLES FOR PULMONARY DRUG DELIVERY

Due to their large surface area and excellent blood supply, the lungs are an attractive alternative route for drug delivery, both for local as well as for systemic action. For a long time, the development of suitable inhalers and to demonstrate effective pulmonary deposition was considered as the most critical step in aerosol drug delivery. However, over the past decade the development of aerosol medicines has made tremendous progress - also grace to the "race" for the first inhalable insulin products, the first generation of which was unfortunately not successful - so that the aerosolization of drugs and their effective pulmonary deposition is nowadays possible by a number of existing technologies. Therefore the scientific problems in pulmonary drug delivery have shifted and are now addressing the question what will happen to aerosol particles "after landings". In order to improve drug absorption across the alveolar air-blood-barrier, novel carrier systems and excipients might be necessary, in order to achieve a better performance for systemic pulmonary peptide/protein delivery or to target nucleotide based drugs e.g. to cancer cells in the lung. In order to achieve long-term controlled relase even of locally acting drugs in the respiratory tract, the typically very rapid mucociliary or macrophage clearance of particles must be controlled, but without impairing the lungs important defence mechanisms. In this context, pharmaceutical nanotechnology again holds a lot of promise.

Starting with the development of a first cell culture model of human alveolar epithelial type-I like cells in primary culture [6, 7] we have recently focused on the development of cell-compatible impinger systems which allow to study both depositon and subsequent drug absorption of pharmaceutical aerosol formulations at the pulmonary epithelial barriers [8]. In an attempt to better understand critical factor of mucociliary particle clearance, we have developed and pre-validated a new in-vitro model based on the trachea of chicken embryos. Finally, we have been involved in some collaborative project to deliver telomerase inhibiting antisences oligonucleotides to lung cancer cells. For this purpose, promising first results could be obtained based on nanoparticles made of positively charged chitosan coated PLGA nanoparicles [9, 10].

References

1. Lamprecht A, Schaefer U, et al., Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa. Pharmaceutical Research, 18: 788-793(2001).
2. Lamprecht A, Ubrich N, et al., Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. Journal of Pharmacology and Experimental Therapeutics, 299: 775-781(2001).
3. Luengo J, Weiss B, et al., Influence of nanoencapsulation on human skin transport of flufenamic acid. Skin Pharmacology and Physiology, 19: 190-197(2006).
4. Lademann J, Richter H, et al., Nanoparticles - An efficient carrier for drug delivery into the hair follicles. European Journal of Pharmaceutics and Biopharmaceutics, 66: 159-164(2007).
5. Stracke F, Weiss B, et al., Multiphoton microscopy for the investigation of dermal penetration of nanoparticle-borne drugs. Journal of Investigative Dermatology, 126: 2224-2233(2006).
6. Elbert KJ, Schaefer UF, et al., Monolayers of human alveolar epithelial cells in primary culture for pulmonary absorption and transport studies. Pharmaceutical Research, 16: 601-608(1999).
7. Fuchs S, Hollins AJ, et al., Differentiation of human alveolar epithelial cells in primary culture: Morphological characterization and synthesis of caveolin-1 and surfactant protein-C. Cell and Tissue Research, 311: 31-45(2003).
8. Bur M and Lehr CM, Pulmonary cell culture models to study the safety and efficacy of innovative aerosol medicines. Expert Opinion on Drug Delivery, 5: 641-652(2008).
9. Ravi Kumar MNV, Bakowsky U, et al., Preparation and characterization of cationic PLGA nanospheres as DNA carriers. Biomaterials, 25: 1771-1777(2004).
10. Nafee N, Taetz S, et al., Chitosan-coated PLGA nanoparticles for DNA/RNA delivery: effect of the formulation parameters on complexation and transfection of antisense oligonucleotides. Nanomedicine: Nanotechnology, Biology, and Medicine, 3: 173-183(2007).

SL002

Is there a need for nanotechnology in immunotherapy?

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INTRODUCTION

In all honesty, "nanotechnology", or even "nanobiotechnology", or any surrogate thereof has been around in pharmaceutical sciences and drug delivery for the last almost 40 years. The fundament for this technology probably has been laid even before, in the early last century by Paul Ehrlich and other visionary scientists. In his hypothesis of the (in)famous "magic bullet", an idea said to be inspired by the visit of the opera "Der Freischuetz", Ehrlich described a delivery system able to deliver specifically its cargo to the tissue afflicted. In his Nobel award speech, he also hypothesized on the interaction of ligands with proteinaceous structures present in cell membranes to be exploited for targeting and enhanced intracellular uptake. Today, "nanotechnology", probably best described as the directed manipulation of all things small (< 1 m), has experienced a renaissance, or better yet, the discovery by a range of scientific and medical disciplines, regulatory authorities, and funding bodies. The former, of course, have had the greatest import on this development, and set in motion the two latter. The reason for this is the astounding increase in knowledge of the world at this level. Microscopy, not at least atomic force microscopy (AFM), has given us the opportunity to examine and manipulate systems at the nanoscale. On the biological side, and through the development of genomics, proteinomics, molecular and systems biology, to name a few, we now can begin to understand the mechanisms involved in processes on the cellular, cellular compartmental, and molecular level, in healthy and diseased states. Moreover, by combining "nanotechnological" and "biological" knowledge and analysis methods, we can attempt to modify drug delivery systems to show an improved pharmacokinetic profile and to better interact with their target tissue.

NANOTECHNOLOGY AND THE IMMUNE SYSTEM

Today, we are experiencing a changement of paradigm when it comes to using nanoscale particulate systems for the delivery of therapeutic agents. With the advent of the production of nanoparticles at an industrial scale, and their use not only in pharmaceuticals, but also convenience products, toxicological aspects have come to the fore. These include the risks of penetration of such small particles through biological barriers, represented by epithelia and endothelia including the blood brain barrier (BBB). This is in stark contrast to, or maybe just the flipside of, the work done in pharmaceutical research of the last decades, where sincere effort has been invested to arrive at exactly this overcoming of biological barriers. Especially the penetration of the BBB has been in the focus of studies aiming to deliver actives into the brain. Another aspect of particulate drug delivery has been the modification of such delivery system to avoid premature interaction with the immune system, e.g., the reticuloendothelial system (RES), to provide for an increase in retention time in systemic circulation and an altered tissue distribution. Nanotechnology at this stage is exemplified by alteration of the surface of particulate carriers, by coupling polymers (surfactants, polyethylene glycole) through covalent binding or adsorption. Targeting to specific cells is then provided by functionalization with targeting moieties, such as monoclonal antibodies, or antibody fragments, or by selective adsorption of plasma proteins to the particle surface. Also here, we are experiencing a change of paradigm in that studies aimed at targeting specific cells of the immune system are being performed, using nanoparticles specifically altered for this purpose. The aim of these studies is the delivery of vaccines and adjuvants to the immune system, resulting in protective immunity or the resolution of an established disease by activating the host's immune system. Vaccination, from this perspective, is eliciting an artificial infection to raise the awareness of the immune system against potential or factual antigens.

Of course, also this idea is not a very recent one. Nature has, over the period of a couple of eons, developed very simple, yet sophisticated and effective "delivery systems" that meet the requirements set forth in the above said. These systems – viruses – are indeed Nature's best (and worst) delivery systems. Viruses are ultramicroscopic disease-producing entities of no cellular organisation, having no metabolic machinery, are unaffected by antibiotics, and are able to alter their phenotype by mutation. In order to develop vaccine delivery systems – using nanotechnology – one might venture into exploring the reasons why viruses are indeed immunogenic.

In my view, there are four reasons why that is the case, and we may have a blueprint for the development of effective artificial vaccine delivery systems in our hands:

VIRUSES ARE PARTICLES

Viruses are take up by antigen-presenting cells (APC), depending on their properties (size, shape,…), which triggers activation of the cellular immune response.

VIRUSES SHOW REPETITIVE STRUCTURES

As viruses have only limited genetic information, they express only a few proteins on their surface, in repetitive sub-units. This structural feature is supposed to be directly activating B-cells and the development of specific T-cell independent immunoglobulin (Ig) M.

VIRUSES ACTIVATE THE IMMUNE SYSTEM THROUGH PRR

Pattern-recognition receptors (PRR) are expressed on various cells of the immune system, and epithelial cells. Conserved throughout evolution, they are able to recognize viral surface patterns specifically, and activate the immune system, forming a first line of defense against infection and bridging adaptive and acquired immunity.

VIRUSES REPLICATE

The exploitation of the host cells' metabolic machinery enables viruses to replicate and thus sustain antigen exposure of the host's immune system. It was shown that the size of the T-cell memory pool directly corresponds to the time of exposure.

Taken these viral properties into account, can we apply nanotechnological methods to copy and include them into nanoparticulate vaccine delivery systems? This question will be discussed and exemplified in this presentation.

SL003

The manufacturing techniques of various drug loaded polymeric nanoparticles

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INTRODUCTION

Nanoparticles composed of polymeric materials have been extensively investigated for their use in delivery and controlled release of low molecular drugs, peptides, and nucleotides via oral, topical, and parenteral routes. A number of different polymers, both synthetic and natural, have been utilized in formulating biodegradable nanoparticles. While synthetic polymers have the advantage of sustaining the release of the encapsulated therapeutic agent over a period of days to several weeks compared to natural polymers which have a relatively short duration of drug release, they are in general limited by the use of organic solvents and relatively harsher formulation conditions. Polymers such as polyglycolic acid (PGA), polylactic acid (PLA), polylactic-glycolic acid (PLGA), poly(e-caprolactone) (PCL), chitosan, albumin, gelatin and alginate are the most common types used for the production of nanoparticulate carrier systems, although there is a host of other novel polymers that have been developed to improve site-specific delivery, controlled release, and drug-loading capabilities.

There are several methods that have been employed to load active molecules into polymeric nanoparticles and selection of one over the other depends on the nature of the polymer and the properties of the drug. They can be classified into two main categories according to wheather the formulation requires a polymerization or is achived directly from a macromolecule or preformed polymer. Polymerization-based methods may present some drawbacks and limitations such as inadequate biodegradability properties of the product and the presence of toxic residues. In order to circumvent those limitations nanoparticles can be prepared directly from preformed syntetic or natural polymers.

SYNTHETIC POLYESTER NANOPARTICLES can be produced using two different approaches.

Emulsification-based methods are based on the emulsification of an organic solution of the polymer in an aqueous phase containing surfactants, stabilizers, or electrolytes, and subsequent precipitation of the polymer by removal of the organic solvent. Some methods involve volatile and water-immiscible solvents which can be extracted by simple evaporation, leading to polymer precipitation (emulsification-solvent evaporation method). In other methods, partially or fully water miscible organic solvents are used and polymer precipitation occurs as a result of controlled diffusion process (salting out and emulsification-diffusion methods).

- *Emulsification/solvent evaporation method* involves the dissolution of the drug and polymer into a volatile organic solvent. The solution is then emulsified into a nonsolvent (aqueous phase with surfactant or stabilizer) using high-energy homogenization to form an O/W emulsion. The extraction of the solvent from the nanodroplets is achived by evaporation. The polymer precipitates leading to the formation of nanospheres. This method provides nanoparticles with high drug-loading efficiency, although it is limited to lipophilic drugs that are soluble in the same solvent as the polymer. Limitations are imposed by the scale up of the high energy requirements in homogenization and the use of toxic chlorinated solvents.
- *Double emulsion technique* is a modification of emulsification/solvent evaporation method and can be employed to encapsulate water-soluble drugs. An aqueous drug solution is emulsified in an organic polymer solution by sonication. This pre-emulsion is then added to an aqueous phase containing PVA as stabilizer, resulting in a W/O/W emulsion which is sonicated again, diluted and finally introduced in a rotavapor to remove the solvent.
- In the *salting-out technique* a water-miscible solvent is used instead of the chlorinated solvents. An aqueous phase containing PVA as a stabilizing agent is added under vigorous stirring to an acetone solution of polymer. The miscibility of both phases is prevented by the saturation of the aqueous phase with electrolytes (magnesium acetate, magnesium chloride). The addition of the aqueous phase is continued until a phase inversion occurs and an o/w emulsion is formed. Then, a sufficient amount of water is added to allow complete diffusion of acetone into water, leading to polymer precipitation in the form of nanospheres. Having minimal affinity for the aqueous phase the drug remains within the organic phase, leading to very efficient entrapment in nanospheres. The use of acetone and large amounts of salts may raise some concern on recycling of the salts and compatibility with active compounds.
- *Emulsification-diffusion method* involves the use of benzyl alcohol as organic solvent which is partial miscible with water (1:25 (m/v)). An aqueous phase containing a stabilizing agent is added to a solution of the polymer in benzyl alcohol under mechanical stirring. A two-phase system is formed and an O/W emulsion is obtained upon complete addition of the aqueous phase. Then, the emulsion is diluted with a large amount of water in order to overcome the miscibility ratio of benzyl alcohol. The precipitation of the polymer occurs as a result of the diffusion of benzyl alcohol into water, leading to the formation of nanospheres.

Direct precipitation of the polymer upon addition of a nonsolvent is a second approach.

- *Nanoprecipitation* involves the use of an organic solvent that is completely miscible with the aqueous phase, typically acetone.The polymer precipitation is directly induced in an aqueous medium by progressive addition of the polymer solution under stirring. After nanoparticle formation, the solvent is removed by vaporization under reduced pressure. The usefulness of this method is limited to drugs that are highly soluble in polar solvents, but only slightly soluble in water, to avoid extensive loss of the drug during the solvent diffusion. In general, this method has to be carried out with low concentrations of polymer in the organic phase.

NANOPARTICLES FROM NATURAL MACROMOLECULES

Among the natural macromolecules available for the manufacture of nanospheres, proteins such as albumin and gelatin, as well as polysaccharides like alginate or agarose have been evaluated. Two main manufacturing techniques have been reported:

- *The emulsification technique* is based on the formation of a w/o emulsion and subsequent heat denaturation or chemical cross-linking of the macromolecule. It is especially suitable for the incorporation of hydrophilic drugs. An aqueous solution of albumin is emulsified at room temperature in a vegetal oil and homogenized. Once a high degree of dispersion is achieved, the emulsion is added dropwise to a large volume of preheated oil under stirring. This leads to the immediate vaporization of the water contained in the droplets and to the irreversible denaturation of the albumin which coagulates in the form of solid nanospheres. The suspension is then allowed to cool down and submitted to several washings using large amounts of organic solvent for complete removal of the oil. This purification step represents a main drawback in terms of manufacturing wastes. In addition, the hardening step by heat denaturation may be harmful to heat-sensitive drugs. To circumvent this latter problem, the use of a crosslinking agent was proposed for the chemical hardening of the albumin nanodroplets.
- *Phase separation process* (coacervation or controlled desolvation methods) in an aqueous medium is the second approach. Gelatin and albumin nanospheres can be produced by the slow addition of a desolvating agent (neutral salt or alcohol) to the protein solution. Upon this addition, a progressive modification of the protein tertiary structure is induced leading to the formation of protein aggregates. The nanospheres are obtained by subsequent crosslinking of these aggregates with glutaraldehyde. It is important to maintain the system at the point just before coacervation is initiated, otherwise large aggregates are formed. Phase separation could be also induced by changes in the pH or temperature, or by addition of appropriate counterions in the protein or polysaccharide aqueous solution. The alginate gelation was induced by divalent cations ($CaCl_2$) and the resulting gelated nanospheres were subsequently stabilized by polyelectrolytic complexation with a polyamine. The phase separation-based methods can be considered less appropriate to incorporate highly water-soluble drugs because they interact more strongly with water than with the macromolecule and are readily washed out of the particles. The major concerns with these techniques involving natural macromolecules lie in the necessity for using hardening agents that may react with the drug and may increase the toxicity of nanoparticle formulations.

In addition to the presented review of different methods for nanoparticle preparation some of our experimental results will also be discussed during the presentation.

SL004

Microparticles and microcapsules – still hot or too big in the Nanoera?

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Microparticles and microcapsules have a long history in drug delivery. However, during the last years, the main focus was on nanoscaled drug delivery systems (NDDS) and "nano" has become a commonly used word (nanotechnology, nanobiotechnology, nanomedicine…). Some of the drug delivery systems, which were "colloidal drug carriers" in former times, are now "nanocarriers". The question arises, whether or not "micro" is too big in the nanoera.

The talk will compare nano- and microscaled drug delivery sytems with respect to

1. size dependence of their physicochemical properties
2. used materials
3. production procedures
4. characterisation methods
5. possibilities of controlled release
6. size dependent interaction with biological enviroments
7. in vivo fate
8. examples of market products

Downsizing from the micro- to the nanorange may lead not only to an increased surface area, but also to different shapes (spherical lipid microparticles but platelet shaped lipid nanoparticles) and degradation pathways. For example, our data indicate that, PLGA-stabilised oily nanocapsules are rapidly digested by Pankreatin.

Special attention will be paid to the possibilities of controlled release, which are very limited for nanoscaled drug delivery systems even in the solid state due to the very short diffusion length. Theoretical considerations and experimental data will be presented on release processes from nano- and microparticles and -capsules.

In summary it can be concluded that microparticles and microcapsules should not be neglected in the nanoera. Although "Nano" is a real hot topic and the key word for many grants, it might not be the best solution in any case. It has to checked if "Micro" does offer similar or even better opportunities. Special care has to be taken in the discussion of controlled release applications from nanoparticles, because artifacts are not uncommon in the literature.

SL005

Practical examples and applications of microparticles

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INTRODUCTION

Microparticulate drug delivery systems, such as microcapsules and microspheres, are widely used in pharmacotherapy for a number of applications such as immediate or controlled delivery for oral or parenteral administration. They are usually spherical, micrometer-sized particles in the size range between 1 to 2000 micrometers. Their advantages over single unit drug delivery systems are absence of dose dumping and larger surface area. The use of biodegradable substances, such as polyesters, alginate and chitosan, has further benefits for safety (1, 2). Microcapsules are also micrometer-sized particles, outwardly similar to microspheres, but with a distinguishable core and shell. The active ingredient is usually located in the core and the shell is usually formed from polymeric material.

A wide variety of methods can be used for the preparation of microparticles. The most common include polymerization of monomers, solvent displacement (either by solvent evaporation or the addition of larger volume of outer phase which is partially miscible with the solvent from inner phase), spray drying, and coacervation.

Drug release form microparticles is strongly influenced by the materials used for the preparation of the microparticles. A desired dissolution profile can be achieved by proper selection of material or material mixtures. Materials used for microparticles preparation can be either synthetic, natural or modified natural materials. Further they can be either biodegradable or non-biodegradable with former being suitable for oral and parenteral application and latter for oral application. The most common synthetic materials are polymethacrylates, polyvinylpyrrolidone, various polyesters (e.g. polylactic acid, polyglycolic acid, polylactic/-polyglycolic acid copolymers, polycaprolactone,…) and polyamides. Natural materials include alginate, gelatin, starch, proteins and lipids. Modified natural materials include modified cellulose derivates, chitosan and lipids.

Two examples of microparticles preparation and characterization are described. First example describes preparation of microparticles with a commonly used emulsion solvent evaporation method using a variety of polymers commonly employed for microparticles preparation. Second example describes preparation of microcapsules with liquid core prepared by vibrating nozzle method, which are rarely described in the literature.

PREPARATION OF MICROPARTICLES BY SOLVENT DISPLACEMENT

Microparticles were produced by emulsion solvent evaporation method commonly used to prepare microparticles. Celecoxib, a selective COX-2 inhibitor, primarily used in treatment of osteoarthritis, rheumatoid arthritis and acute pain was encapsulated in microparticles composed of various polyesters, polymethacrylates or cellulose derivatives used alone or in mixtures. Altogether, eleven different polymers and three polymer mixtures were tested. The influence of polymers on microparticle mean diameter, encapsulation efficiency and in vitro and in vivo celecoxib release was investigated.

Mean particle diameter was in the range of 11-37 μm which indicates no significant influence of the tested polymers on particle diameter. The size range of similar microparticles found in literature is usually between 1 to 100 μm since the diameter of the microparticles can be easily affected by a variety of processing factors such as surfactant concentration, stirring rate, volume and viscosity of both phases and molecular weight of the polymers. In our case the viscosity of the inner phase and the molecular weight of the polymer did not seem to have a great influence on the particle diameter. Encapsulation efficiency was close to 100% in all cases mostly because of poor solubility of celecoxib in 0.1% PVA aqueous solution used as outer phase. Nevertheless, processing parameters such as the drug/polymer ratio, surfactant concentration and stirring speed also play an important role with regard to encapsulation efficiency of celecoxib. The optimal encapsulation efficiency in our case can therefore also be attributed to good processing parameters.

Considering in vitro release kinetics, microparticles could be divided into drug delivery systems with fast and slow release profiles. Microparticles with fast release profiles released 60-80% of the encapsulated celecoxib in 48 hours without any significant release for the next 7 days. Microparticles with slow release profiles released 10-25% and 15-45% of encapsulated celecoxib in 48 hours and seven days, respectively.

Microparticles prepared with poly-ε-caprolactone, Eudragit® RS and low viscosity ethylcellulose (Figure 1), together with physical mixture of celecoxib with lactose and Celebrex®, were tested *in vivo*. Relative

bioavailability of celecoxib was below 20% in all cases and was probably the consequence of a slow *in vivo* release of celecoxib from microparticles or low wettability in the case of Celebrex® and physical mixture.

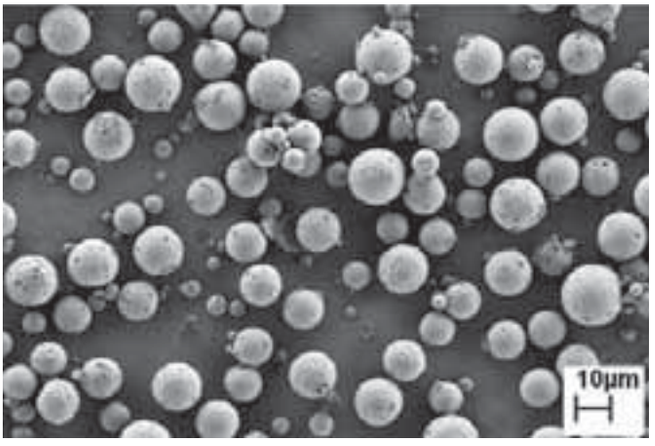

Figure 1: Celecoxib loaded microparticles prepared with emulsion solvent evaporation method and low viscosity ethylcellulose matrix forming polymer.

PREPARATION OF MICROCAPSULES BY VIBRATING NOZZLE METHOD

The purpose of this work was to prepare alginate microcapsules with a liquid self-microemulsifying system (SMES) containing celecoxib in the core with a vibrating nozzle method. An Inotech IE-50 R encapsulator equipped with a concentric nozzle was used to prepare the microcapsules. The vibrating nozzle method is often used for preparing alginate beads, since it allows a high rate of production of uniform sized beads with mean diameters below 300 mm. The basic principle is the breakup of laminar liquid jet into equally sized droplets by a superimposed vibration. The resulting droplets are usually hardened by incubation in the hardening solution by ionotropic gelation (i.e. gelation of sodium alginate solution by the addition of divalent of polyvalent ions). Preparation of microcapsules is possible by the use of concentric nozzle with two separate liquid feeds.

The SMES was shown to significantly increase celecoxib solubility over that of the pure drug. Doubling the amount of SMES the solubility of celecoxib increased by eightfold, resulting in 400-fold increase in solubility over that of the pure drug.

Preparation of microcapsules with liquid SMES core proved to be challenging due to quick mixing of the core phase with shell forming phase and subsequent leaking of the core phase. In order to increase the amount of core phase that could be incorporated into microcapsules, the core phase was saturated with $CaCl_2$ to promote shell hardening as soon as the microcapsule was formed. Flow speeds of both phases were optimized to the lowest possible settings to decrease the mixing of the both phases. Lactose and sodium chloride were included in the shell forming phase in addition to sodium alginate to decrease leaking of the core phase. After formation, microcapsules were coated with chitosan to further decrease core leaking. Finally, microcapsules were dried in a fluid bed apparatus.

Microcapsules prepared with celecoxib dissolved in liquid SMES exhibited distinct core vesicles containing liquid SMES (Figure 2). By modifying the SMES, shell phase and including an additional chitosan coating, drug loading in the range from 17 to 33% could be achieved with the encapsulation efficiency ranging from 60 to 82%. Compared to the previous report, drug loading capacity was significantly improved, enabling the formulation of suitable sized dosage forms (3). Alginate microcapsules loaded with SMES and celecoxib showed increased dissolution rate of celecoxib over that of alginate microcapsules loaded with celecoxib or that of the celecoxib alone.

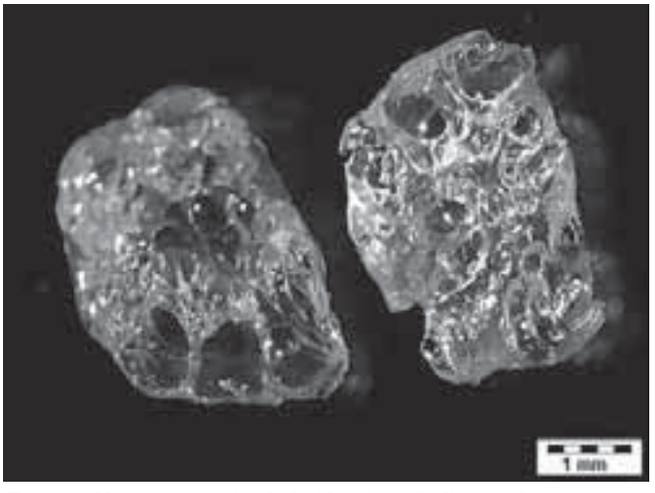

Figure 2: Microcapsules loaded with celecoxib dissolved in liquid self-microemulsifying system.

References

1. Kas HS. Chitosan: properties, preparations and application to microparticulate systems. J Microencapsul. 1997; 14(6): 689-711.
2. Freiberg S, Zhu XX. Polymer microspheres for controlled drug release. Int J Pharm. 2004; 282(1-2): 1-18.
3. Homar M, Suligoj D, Gasperlin M. Preparation of microcapsules with self-microemulsifying core by a vibrating nozzle method. J Microencapsul. 2007; 24(1): 72-81.

SL006

Pellets – A universal tool for oral drug delivery

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Pellets are the largest particles, which are covered in the Symposium. The mean particle size is in the range from 0.2 to 2 mm while the particle size distribution is narrow compared with conventional granules. Pellets have more or less a spherical shape and a smmoth surface. The main use is oral drug delivery.

Pellets as multiparticulate systems are advantageous compared to monolithic solid dosage forms, if they provide some biopharmaceutical functionality. This can be enteric resistance or many different ways of a modified release.

Many techniques are available for the production of pellets. Depending on the technique used the resulting pellets are homogeneous or heterogeneous. In many cases pellets are coated to achieve the desired release profile.

Extrusion/ spheronisation will be discussed in more detail. Pelletisation aids are crucial for the formation of pellets. Microcrystalline cellulose (MCC) is an established pelletisation aid since nearly 50 years. However, the use of MCC is connected with some problems like the missing disintegration and the resulting slow dissolution of low soluble drugs. Recently, alternative pelletisation aids have been proposed. Some of these materials will be critically evaluated.

SL007

Analytics of biopharmaceutics in multiparticulate systems

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The effective delivery of therapeutic proteins to the site of action is of great importance in achieving an effective therapy. In this case nanotechnology seems to offer the unique delivery approach. Nanometer-sized particles have been developed for the delivery of various low molecular drugs, however, for protein drugs specific formulation has to be applied to preserve their therapeutic activity. Liposomes or polymeric nanoparticles can protect the protein drug from premature degradation and control its release at the site of action enhancing therapeutic efficacy and reducing undesirable side effects. Specific delivery of drug-incorporated nano-vehicles can be achieved via active targeting by introducing ligands that are recognised uniquely by receptors or certain other proteins. These ligands are usually monoclonal antibodies. Besides analytical tools used for characterisation of "classical" parameters of nano-vehicles, such as size, zeta potential and morphology, protein containing nano-vehicles demand the tools for analysis of protein content, protein structure and biological activity, coating efficacy, protein-protein interactions, cell internalisation, etc. For this purpose the application of surface plasmon resonance (SPR), fluorescent microscopy, flow cytometry and other immunological methods will be discussed. Nano-vehicle system can be efficient also in the delivery of drugs to the intracellular targets due to the enhanced endocytosis, typical for highly proliferating cells. As an example, poly(lactide-co-glycolide) PLGA nanoparticles, incorporating active protease inhibitor and labelled with monoclonal antibody, specific for tumour associated antigens expressed on the surface of breast epithelial tumour cells will be presented. The new delivery system was tested on various invasive breast tumour cells and co-cultures of breast tumour cells and enterocytic Caco-2 cells or monocyte/macrophage cells. Our results clearly showed that nanoparticle system, incorporating protease inhibitor and tumour specific monoclonal antibody, solely bound to tumour cells, internalized the cells rapidly inactivating intracellular proteolytic activity. On this way the invasiveness of tumour cells was significantly impaired.

References:

1. Obermajer, N., Kocbek, P., Repnik, U., Kužnik, A., Cegnar, M., Kristl, J., Kos, J. Immunonanoparticles – an effective tool to impair harmful proteolysis in invasive brast tumour cells. FEBS Journal 274 (2007) 4416-4427.
2. Kocbek, P., Obermajer, N., Cegnar, M., Kos, J., Kristl, J. Targeting cancer cells using PLGA nanoparticles surface modified with monoclonal antibody. J. Controlled Release 120 (2007) 18-26.
3. Cegnar, M., Premzl, A., Zavasnik Bergant, V., Kristl, J., Kos, J. Poly (lactide-co-glycolide) nanoparticles as a carrier system for delivering cysteine protease inhibitor cystatin into tumour cells. Exp. Cell Res. 301 (2004) 223-231.
4. Cegnar, M., Kristl, J., Kos, J. Nanoscale polymer carriers to deliver chemotherapeutic agents to tumours. Exp. Opin. Biol. Ther. 5 (2005) 1557-1569

SL008

Critical regulatory issues for new drug delivery systems

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INTRODUCTION

New drug delivery systems encompass all administration routes. The relevant literature in pharmaceutical technology shows that many drug delivery systems have been described during the last 20 years. Such systems were potentially proposed for licensing (if patented) to many pharmaceutical companies. However, when one looks at the new systems available on the pharmaceutical world market, it becomes obvious that many of these systems have never (and will probably never) been marketed. So it is important to wonder the reasons for the gap between the scientific literature and the low number of marketed new drug delivery systems.

REGULATORY ENVIRONMENT

It will be assumed that the new drug delivery systems have potential therapeutic interest and have been patented. This is an important point since many academic systems have not been patented and therefore there is no real interest for any company to market them. At the opposite, pharmaceutical companies generally patent dosage forms whether conventional or innovative. Nevertheless, for patented drug delivery systems, there is still a long road from the concept to the market. The difficulties, from a pharmaceutical point of view, are related to the current guidelines existing in Europe but also in the USA and Japan (just to mention the most important world areas).

Basically, any company wanting to market a new drug delivery system will have to deal with guidelines on excipients, pharmaceutical development, manufacturing, control of finished product at release but also stability which may be more critical for these systems. It may also be of interest to be aware of the relatively new guideline on early clinical trials.

The following guidelines need to be well known in case of interest for new drug delivery systems:

- Excipients in the dossier for application for marketing authorisation of a medicinal product (III/3196/91 [3AQ9a])
- Note for guidance on pharmaceutical development (ICH Q8 EMEA/CHMP/167068/2004)
- Annex II to note for guidance on process validation, Non standard processes (CPMP/QWP/848/96)
- Specifications : test procedures and acceptance criteria for new drug substances and new drug products (CPMP/ICH/367/96 corr)
- Stability testing of existing active substances and related finished products (CPMP/QWP/122/02 Rev 1)
- Requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials (CHMP/QWP/185401/2004).

Of course, many other guidelines may have an impact on the development of the new drug delivery systems and are not reported here. For instance there are guidelines on residual solvents, sterilisation, pressurised meter dose inhalation products ...

CRITICAL REGULATORY ISSUES

The critical issues may be found in the previously mentionned guidelines. However the first question may be to specifically define what does new drug delivery system mean? Many of the new delivery systems are injectable systems (microparticles, stealth liposomes, implant whether biodegradale or not...) but also topical dosage forms such as iontophoretic patches.

If the new delivery system has demonstrated efficacy during clinical trials, the major difficulty will be to further demonstrate that the quality of the system is well under control. Obviously, this should be demonstrated in the Common Technical Document where the different quality points will be discussed in part 3.P (finished product). The key point will be Pharmaceutical Development. An assessor from any health authority should find the justification of the composition but also a clear description of the critical manufacturing issues and how they can be controlled. As stated in the guideline the aim of the pharmaceutical development is to design a quality product and its manufacturing process to consistently deliver the intended performance of the product. The information and knowledge gained from pharmaceutical development studies and manufacturing experience provide scientific understanding to support the establishment of the design space, specifications, and manufacturing controls. Design space is a relatively new tool which should be understood as the multidimensional combination and interaction of input variables (e.g. material attributes) and process parameters that have been demonstrated to provide assurance of quality.

Among the critical issues, many can be raised because of new excipients with tailor-made properties. This was for instance the case for the pegylated lipids which are confering the liposomes their stealth properties. In this case, it is necessary to refer to the guideline about new excipients. For new excipients not described in the European Pharmacopoeia or in the Pharmacopoeia of a member state, a dossier should be established containing the same data as required for new active substances. In such a case, data concerning the toxicology of the new excipients should be presented according to the dosage form and the route of administration of the medicinal product. It can be easily understood that such a requirement (actually fully justified) may slow down the arrival on the market of very innovative dosage forms based, for instance, on what is called intelligent excipients. Once the composition has been justified by a correct pharmaceutical development, it will still to be proven that the new drug delivery system can be maufactured routinely with critical issues well under control. Therefore the part devoted to the manufacturing process and its validation is also critical.

Most of the time, new drug delivery systems will fall under the validation guideline on non-standard processes. The manufacturing process will have to be validated on 3 full scale size batches according to numerous parameters. It has to be noted that specialised dosage forms such as parenteral depot based on biodegradable polymers, liposomal preparation or micellar preparations are considered as non conventional dosage forms. More surprisingly, the guideline on non-standard processes also include prolonged release preparations, suspensions, emulsions as specialised dosage forms needing a full validation on the claimed industrial batch size.

When all these critical steps are overcome, control of the finished drug product should be easier although new tests may have to be elaborated. For instance, one could wonder how it is possible to measure «stealth properties» with an in vitro test! Nevertheless, since all the critical issues and steps will be known at the time of setting the specifications, there should not be major problems at this stage of the marketing authorisation file.

Stability studies are very well defined and the applicant should follow the ICH guidelines with regards to both temperature and humidity conditions. The problem will not be a regulatory one but more a problem linked to the dosage form and its susceptibility to aging. For these new drug delivery systems, shelf-life may be reduced with regards to conventional dosage forms which is, again, another problem before reaching the market. For instance in the case of liposomes, the first ones to reach the market were freeze-dried because they were not stable enough as a ready-for-use suspension. Consequently, the applicants had to prove that the diameter was still the same before and after lyophilization. Other dosage forms have to be stored at minus 20°C because of polymer degradation and this is definitely not in favour of easy marketing in every country.

CONCLUSION

Launching a new drug delivery system on the market is not something which is imposible. However, it will be a long road from the concept to the validated batches. On the other hand, health authorities are always ready to help in developping innovative dosage forms. The key point is to know early enough if a development should be continued or stopped with regards to guidelines and experience. Definitely new drug delivery systems are very risky in terms of final success but the medical world definitely needs innovative dosage forms for all the biotech drugs and this is a great opportunity for joined research project betwen academy, industry and health authorities all over the world.

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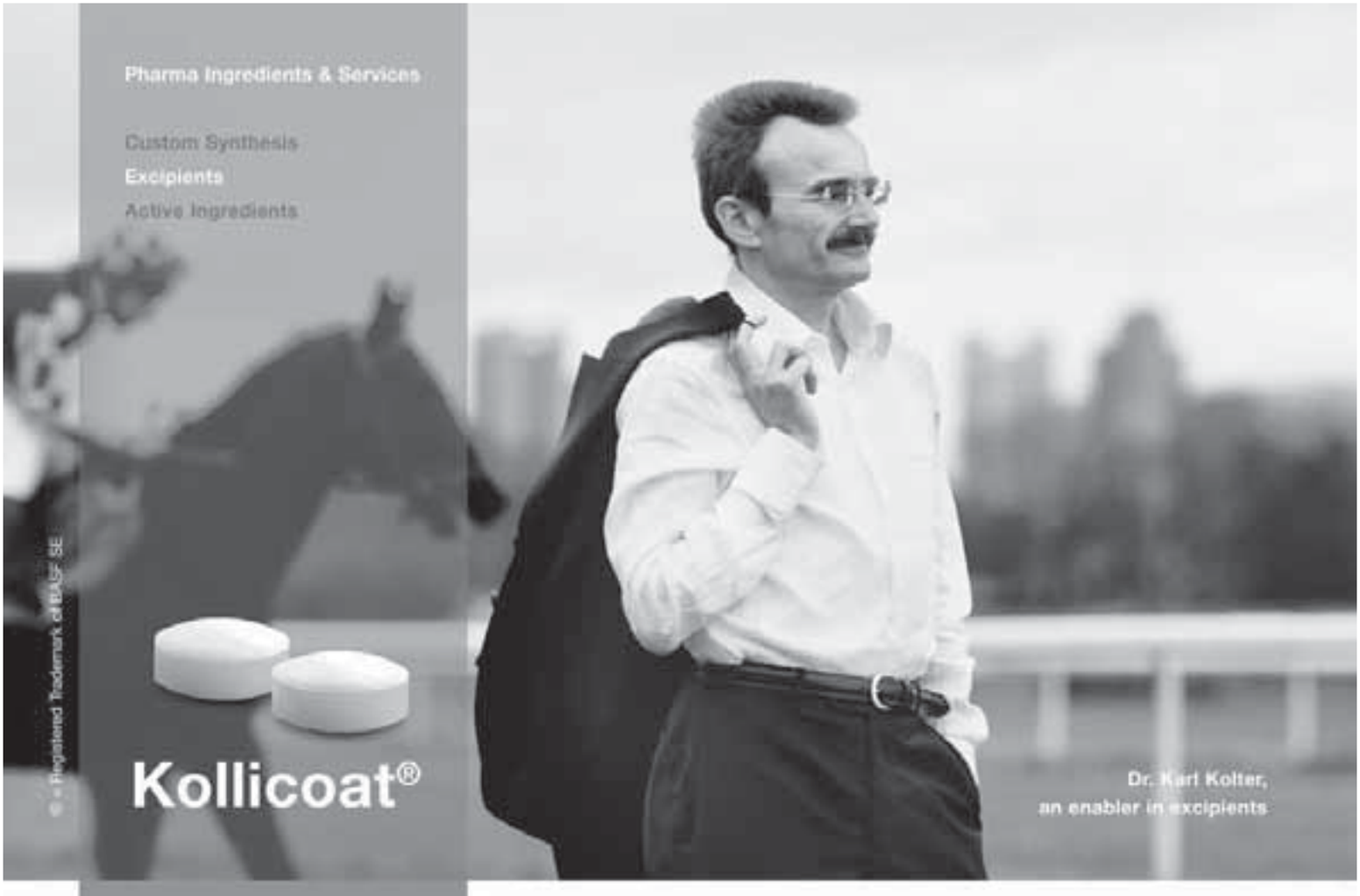

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OP001

Protamine-oligonuclotide nanoparticles as drug delivery system for vasoactive intestinal peptide

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INTRODUCTION

In recent years nanoparticle formulations have attracted considerable attention as drug delivery systems. Among them are biodegradable nanoparticles (NP) consisting of oligonucleotides (ON) and protamine as developed by . Studies on these - so called "proticles" - concerning particle size, zeta potential and cellular uptake lead to promising results. Thus "proticles" have been selected as drug carriers for the development of a pulmonary depot system. Our research is focused on a peptide called Vasoactive Intestinal Peptide (VIP), which is deployed for the treatment of primary pulmonary hypertension (2). VIP is a neuropeptide that acts as a potent systemic and pulmonary vasodilator. However the disadvantage of using VIP in therapy is the short half life of the peptide after pulmonary application. To overcome this limitation we entrapped VIP into the NP matrix and thus protect the peptide against rapid enzymatic degradation. For this purpose we used NP which are prepared by self-assembly of ON, VIP and protamine in aqueous solution. The characterization of this VIP-loaded NP focused on particle size and zeta potential as well as VIP loading and VIP release out of the NP matrix. Furthermore the pharmacological VIP response on lung arteries as well the cellular uptake has been investigated.

MATERIALS AND METHODS

Biophysical Characterization

VIP-loaded Proticles were characterized by the means of Dynamic Light Scattering analysing particle size (hydrodynamic diameter) and surface charge (zeta potential). Additionally, Scanning Electron Microscopy (SEM) images of freeze dried nanoparticle suspensions were used to visualize the shape of the NP.

VIP loading and VIP release

The amount of VIP entrapped into the nanoparticle matrix was quantified by RP-HPLC. Nanoparticle suspensions were centrifuged for 2 h in order to separate the supernatant from the pellet. HPLC-analytics of the supernatant and the pellet was used to quantify unbound VIP.

To simulate pulmonary conditions drug release was investigated in BAL (broncho-alveolar lavage). The quantification of released VIP was also performed by RP-HPLC, however, in order to increase the sensitivity Cy3-conjugated VIP was deployed. Briefly, separation of unbound VIP was carried out followed by lyophilization. Further, the dried proticles were dissolved in BAL and incubated on an orbital shaker (37 °C, 300 rpm). After indicated time periods samples were centrifuged (2h, 4 °C, 20.000xg) and the amount of "released" Cy3-conjugated VIP was measured.

Pharmacological VIP response on lung arteries

The pharmacological effect of VIP loaded nanoparticles was investigated by measuring the relaxation response on rat lung arterial strips in oxygenated organ baths. VIP-loaded proticles were purified by SEC (PD-10 desalting columns) in order to remove free VIP followed by lyophilization to improve storage conditions. Prior to each application the samples were dissolved in the desired amount of water and the resulting proticle suspension was injected into the organ bath after precontraction of lung arteries with phenylephrin.

RESULTS AND DISCUSSION

Biophysical Characterization

Initially, we found a primary complex between ON and VIP which was indicated by the turbidity of the solution and hydrodynamic diameters of the particles of approx. 300 nm. After the addition of protamine resulting in mass ratios (ON:VIP:protamine) of 1:1:0,6, 1:1:0,8 and 1:2:0,6 respectively, the particle size of the proticles decreases slightly (150-250nm). Zeta potential measurements show values between - 13 and - 24mV. SEM images of freeze dried nanoparticle suspensions visualized the shape of VIP-loaded proticles and confirmed data concerning nanoparticle size [Fig. 1].

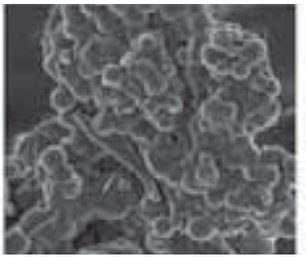

Figure 1: SEM images of VIP-Proticles

VIP loading

The overall amount of VIP in the supernatant, representing free VIP was found to be between 20 and 30 % of the VIP deployed. These data could be confirmed by HPLC-analytics of the pellet after dissolving it with TFA [Fig. 2].

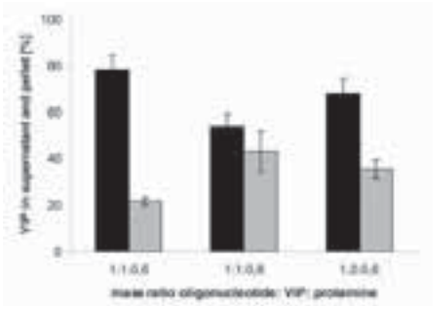

Figure 2: Amount of VIP entrapped into proticles

VIP-release

The release was calculated as percent of total VIP. Two concentrations of VIP- loaded proticles were investigated. Data indicated that between 60 and 87 % of the VIP dosage was released within the first eight hours [Fig.3].

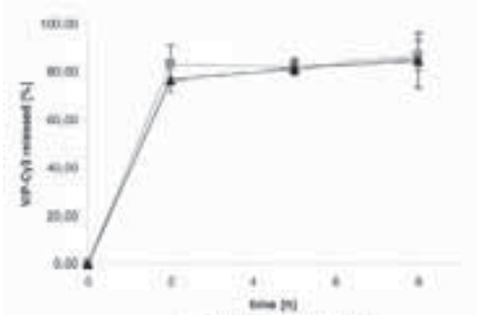

Figure 3: VIP release from nanoparticles (△ 75 g/ml; □ 35 g/ml)

Pharmacological VIP response on lung arteries

About 300 seconds after the addition of the proticles the arteries relaxed by about 30 to 40 %, before the recontraction started. This recontraction was fast when using free VIP (black) and was delayed when VIP was packed into proticles (red) demonstrating a depot phase [Fig. 4].

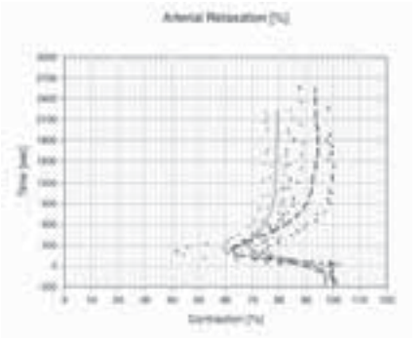

Figure 4: Relaxation of lung arteries

CONCLUSION

The incorporation of VIP into proticles resulted in a drug delivery system with appropriate size and loading capacity. Further satisfying drug release as well as pharmacological VIP-response on arteries could be observed ex vivo. Additionally, cellular uptake studies of VIP-proticles as well as investigations concerning cytotoxicity will be carried out.

References

1. Junhans, M., Kreuter, J. & Zimmer, A. (2000) Nucleic Acids Res 28, E45
2. Petkov, V., mosgoeller, W., Ziesche, R., Raderer, M., Stiebellehner, L., Vonbank, K., Funk, G. C., Hamilton, G., Novotny, C., Burian, B. & Block, L. H. (2003) J Clin Invest 111, 1339-46.

OP002

Formulation and evaluation of new drug candidates in a nanodelivery system

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INTRODUCTION

Breast cancer is the most frequent type of cancer in women and the second most frequent cause of cancer death. Therefore, inhibitors of type 1 17β-hydroxysteroid dehydrogenase (17β-HSD), an enzyme involved in estradiol biosynthesis, would be beneficial to block the local production of estradiol in breast cancer cells and consequently decrease cell proliferation [1]. Since type 1 17β-HSD enzyme is located in the cytoplasm of malignant epithelial breast tumor cells, effective intracellular delivery of type 1 17β-HSD inhibitors is crucial to reduce estradiol concentration in the tumor cells. Unfortunately, many of the drug candidates that exhibit high inhibitory potency are poorly soluble in water and some are also unstable in aqueous medium. Therefore, a suitable delivery system, which will protect the drug and assure its intracellular delivery, is needed. This goal can be achieved using a nanoparticulate formulation, since nanoparticles can enter tumor cells by an endocytotic process, allowing the drug to be released into the interior of the cells, thus contributing to an increase in its concentration near the site of action.

The aim of this research work was to formulate polymeric nanoparticles loaded with new drug candidate, phenyl-3-(3,4,5-trimethoxyphenyl)prop-2-enoate (I-1) or (benzyl-3-phenylprop-2-enoate) I-2, and to

investigate whether incorporation into nanoparticles brings any biological benefit over the free compounds. In addition to physicochemical properties of prepared nanoparticles, internalization of nanoparticles and their effect on cell viability and cell cycle were also evaluated.

EXPERIMENTAL METHODS

Nanoparticle preparation. Poly(ε-caprolactone) (PCL) nanoparticles loaded with active compound I-1 or I-2 were prepared using solvent displacement method [2]. 60 mg of PCL was dissolved in 10 mL of acetone using an ultrasound bath and the solution was slowly injected into 50 mL of 0.3% (w/v) aqueous solution of poloxamer 188 under moderate magnetic stirring. The resulting nanoparticle dispersion was stirred for 24 h at room temperature to evaporate the acetone. The nanoparticles were

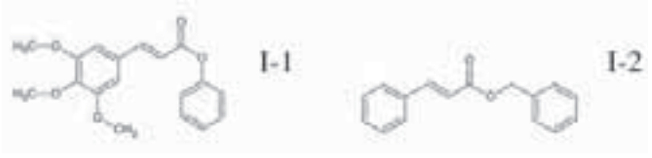

Figure 1: Chemical structures of model drugs phenyl-3-(3,4,5-trimethoxyphenyl)prop-2-enoate (I-1) and benzyl-3-phenylprop-2-enoate (I-2) representing type 1 17β-HSD inhibitors.

trehalose as a cryoprotectant. For preparation of drug- or coumarin-6-loaded nanoparticles the drug (I-1 or I-2) or coumarin-6 was dissolved in acetone along with the PCL before injection into aqueous solution of stabilizer. The drug to polymer weight ratio was 1:9.

Nanoparticle characterization. Particle size was measured by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, Worcestershire, UK). The surface was determined using the same equipment. The morphology of nanoparticles was visualized by scanning electron microscopy (SEM) (Supra 35 VP, Zeiss, Germany). The total amount of drug in the nanoparticle dispersion was determined by HPLC analysis.

Cell culture. The hormone-sensitive breast cancer cell line T-47D was purchased from European Collection of Cell Cultures (Salisbury, UK). Cells were cultured in supplemented DMEM medium in a humidified atmosphere of 5% CO_2 in air, at 37 °C. Growth medium was additionally supplemented with a physiological concentration of estrone (5 nM), which is the substrate for type 1 17β-HSD, the enzyme studied in this work.

Viability and internalization assay. The effect of free drug, plain and drug loaded nanoparticles on T-47D breast cancer cells was evaluated using CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (Promega, Madison, WI, USA) according to the manufacturer's instructions. Cell viability was expressed as percentage of the absorbance of untreated cells.

Cell cycle analysis. The distribution of DNA in the cell cycle was studied by flow cytometry (FACSCalibur, Becton Dickinson, Inc., USA).

RESULTS AND DISCUSSION

The new, very poorly soluble and unstable in aqueous media, inhibitors were incorporated in PCL nanoparticles in our research work. All nanoparticle formulations had mean diameters in the range of 130 to 170 nm with low polydispersity. The SEM image revealed their regular spherical shape, as well as a range of diameters. Incorporation of I-1 into the PCL matrix slightly decreased the particle size, compared to plain PCL nanoparticles, while I-2 loading had no significant effect on mean particle size. All PCL nanoparticles showed negative surface charge around – 29 mV. Drug loading was high for both active compounds, however, it was slightly higher for more hydrophobic compound I-2 (86.1%) compared to I-1 (78.9%).

Internalization assay using T-47D breast cancer cells showed uptake of nanoparticles and their intracellular localization throughout observation time (up to 11 days after addition to the cell culture) (Fig. 2). Furthermore, intracellular delivery of new *trans*-cinnamic acid esters I-1 and I-2 incorporated in polymeric nanoparticles effectively decreased proliferation of T-47D breast cancer cells (Fig. 3), whereas inhibitors added in a suspension or plain nanoparticles (in concentration up to 400 μg/ml) were unable to exert such an effect. Cell cycle analysis demonstrated that treatment of cells with drug-loaded or plain nanoparticles had no effect on the cell-cycle distribution (Fig. 4). This study revealed that when inhibitor was incorporated in nanoparticles, its transport through the cell membrane was greater than when applied in suspension. It can therefore be assumed that incorporation of inhibitors in nanoparticles increases their biological effect.

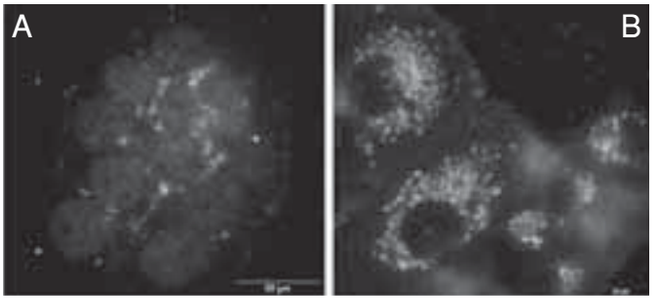

Figure 2: Internalization of coumarin-6 loaded PCL nanoparticles into T-47D breast cancer cells after 2 h (A) of incubation. Cell nuclei were stained blue using Hoechst 33342 dye. Live-cell imaging of nanoparticle uptake after 8 days of incubation (B).

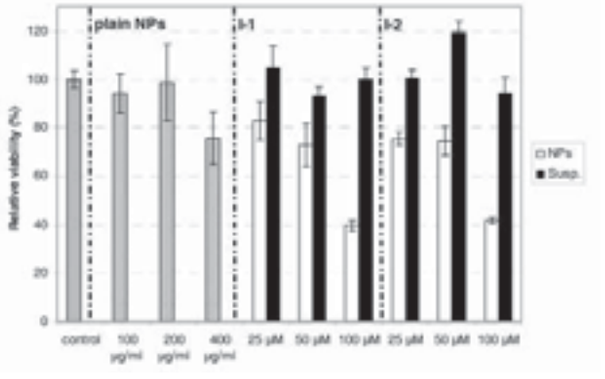

Figure 3: Effect of drug suspension (black columns), plain nanoparticles (striped columns) and drug-loaded nanoparticles (white columns) on viability of T-47D cells after 48 h incubation.

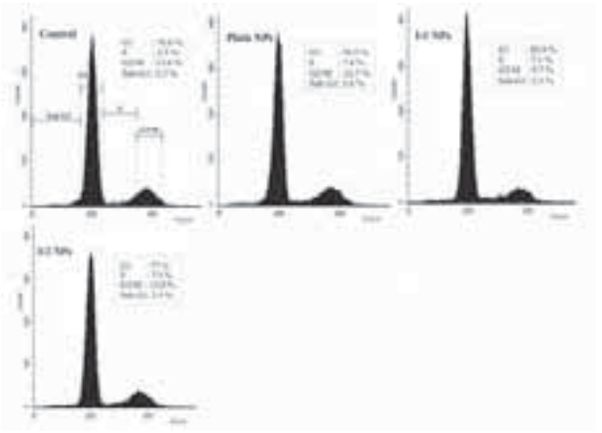

Figure 4: Cell cycle analyses of untreated T-47D cells (Control) or cells treated for 6 days with plain (200 μg/ml) or drug-loaded (50 μM) PCL nanoparticles.

CONCLUSION

We have shown that formulation of a suitable delivery system is necessary to achieve a biological effect. Intracellular delivery of new *trans*-cinnamic acid esters I-1 and I-2 incorporated in polymeric nanoparticles effectively decreased viability of T-47D breast cancer cells, whereas inhibitors added in a suspension were unable to exert such an effect. This effect can be attributed to the drug induced inhibition of type 1 17β-HSD that leads to decreased cell proliferation. Besides drug internalization, PCL polymer protects the incorporated drug against degradation in aqueous medium and enables its sustained release inside the cells.

References

1. P. Brožič et al. Curr. Med. Chem. 15 (2008) 137-150.
2. J.S. Chawla et al. Int. J. Pharm. 249 (2002) 127-138.

OP003

Freeze-drying of squalenoylated nucleoside analogue nanoparticles

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INTRODUCTION

Nucleoside analogues are potent anticancer or antiviral agents that undergo some limitations (rapid metabolism, induction of resistance). In order to overcome them, we have covalently coupled a hydrophobic derivative of squalene to various nucleoside analogues (Gemcitabine, ddI and ddC).[1] These amphiphilic prodrugs spontaneously form nanoassemblies in water.[1] The squalenoylated Gemcitabine nanoparticles have displayed a promising *in vivo* activity.[2] Their original supramolecular organization[3] confers on them several liposome-like rather than polymer nanoparticle-like physicochemical properties.

A major concern with nanoparticulate systems is the control of their colloidal stability, which only lasts a few days in case of our nanoagregates. This shortcoming had to be overcome in order to undertake their preclinical evaluation. We have therefore developed a laboratory scale freeze-drying protocol, readily applicable to various squalenoylated nucleoside analogues.

EXPERIMENTAL METHODS

Preparation of the nanoparticles: Squalenoylated Gemcitabine (SQdFdC), ddI (SQddI) and ddC (SQddC) nanoparticles (NPs) were prepared by nanoprecipitation: an ethanolic solution of the organic compound was added dropwise under vigorous stirring to an aqueous phase (containing the cryoprotectants), after which the ethanol was evaporated using a Rotavapor®.

Lyophilization : 500 μL of nanoparticular dispersion in glass vial were frozen (2H at -25°C or 5 minutes in liquid N_2) and put in a freeze-dryer (pre-equilibrated at -20°C) which chamber was depressurized (~ 5 Pa) for 62 hours of primary drying, followed by 28 hours of secondary drying at 25°C. Formulations were rehydrated with MilliQ water under gentle stirring.

Stability assessment : The NP's size profile was measured by dynamic light scattering. Suspension stability was monitored over the course of 7 days.

In vitro *cytotoxicity assay :* The cytotoxic activity of SQdFdC nanoparticles was assessed on three cell lines (2 variant strains of P388 murine leukaemia, and the J774 murine macrophages) using an MTT assay. IC50 were obtained after a fit to a Hill function.

RESULTS AND DISCUSSION

• Freeze-drying parameters

The first step was to select the appropriate cryoprotectant. The SQdFdC NP's concentration was fixed at 1 mg/mL. Glucose led to a very poor NP redispersion. Alternatively, trehalose at 10% (w/w) yielded acceptable freeze-dried cakes (neither collapse nor shrinkage). After rehydration the complete redispersion was confirmed by Nanosizer analysis. Adding polysaccharides to trehalose (*eg* Dextran 70) worsens the redispersion, most likely through destabilizing interactions with the polar part of our compound.

Different freezing conditions were compared : freezing on shelf at -25 °C for two hours led to slightly smaller and more monodisperse NPs after rehydration than liquid N_2 cooling.

Table 1: Evolution of SQdFdC nanoparticles during the freeze-drying and rehydration steps.
**: rehydration of a lyophilized 2 mg/mL suspension with less water*

[SQdFdC] after rehydration (mg/mL)		1	2	4*
NP mean diameter	Control	113 nm	141 nm	
	Freeze-dried	135 nm	161 nm	165 nm
Polydispersity index		+ 0,6%	- 12,0%	- 12,0%

• Increasing SQdFdC concentration

The above protocol was not suitable for concentrations higher than 2 mg/mL SQdFdC and led to incomplete redispersion. Since concentrations of at least 4 mg/mL SQdFdC are required for *in vivo* experiments, the problem was solved by rehydrating a 2 mg/mL formulation with half the amount of evaporated water (Table 1).

• Stability of the rehydrated nanoparticles

As shown in , the freeze-drying step only led to a ~20% increase of the NP size immediately after rehydration and was even found to stabilize the lower concentrated nanoparticles. Concentrated NPs displayed a better stability after rehydration than diluted ones.

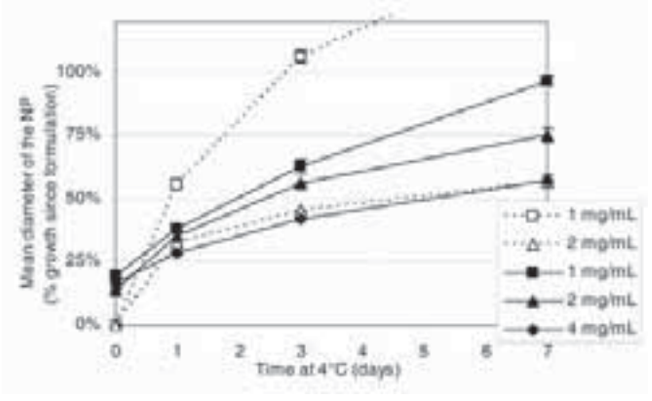

Figure 1: Stability over one week of SQdFdC NPs at different concentrations, freeze-dried (■▲◆) or not (□△)

• *In vitro* cytotoxic activity

We demonstrated that there was no significant loss of cytotoxic activity of the freeze-dried nanoparticles (Table 2).

Table 2: IC50 ratios (non freeze dried / freeze dried) determined on 3 murine cell lines in vitro.

Cell line	P388	P388/adr	J774
IC50 ratio	1,19	0,93	0,96

• Shelf life of the freeze dried formulation

The shelf life of our formulations was compared with the timeframe of the SQdFdC preclinical studies in order to assess the relevance of the process. Freeze-dried NPs were stored at room temperature or at 4 °C for 1 week, 1 month or 4 months before rehydration. Good reconstitution and stability were obtained, even after 4 months, whatever the SQdFdC concentration. An interesting side-result was that the rehydrated NP diameter diminished as a function of the storage time. This experiment also demonstrated that the storage at ambient temperature was preferable.

• Application to other nucleoside analogues

Two other squalenoylated nucleoside analogues under investigation in our laboratory (SQddI and SQddC) can be nanoprecipitated like SQdFdC. Those NPs were freeze-dried with different cryoprotectants and freezing rates. Good results were obtained for both molecules at 1 mg/mL (Table 3). Interestingly, 5% trehalose was sufficient in the case of SQddI. On-shelf-freezing at -25 °C once more proved to be slightly better than the other freezing conditions.

Table 3: Evolution of SQddC & SQddI NPs during the freeze-drying and rehydration steps. Freezing at -25 °C

Nucleoside analogue		SQddC	SQddI	
Trehalose concentration		10%	5%	10%
NP Mean diameter	Control	118 nm	176 nm	176 nm
	Freeze-dried	147 nm	188 nm	190 nm
Polydispersity index		+ 33,3%	- 10,1%	-13,9%

CONCLUSION

We have developed and characterized a freeze-drying protocol allowing SQdFdC nanoparticles to be stored for several months in solid form, without loss of stability or biological activity upon rehydration. This technique can also be applied to other squalenoylated compounds developed in our laboratory. The conception of a successful freeze-drying protocol is an essential milestone in the pharmaceutical development of a nanomedicine. Our results will contribute to the scaling up required for further preclinical studies of nanoparticular squalenoylated nucleoside analogues.

References

1. Couvreur P. *et al*. Nano Lett. 6: 2544-48 (2006)
2. Reddy L.H. *et al*. J. Control. Release. 124: 20-27 (2007)
3. Couvreur P. *et al*. Small. 4: 247-53 (2008)

OP004

Chitosan particles to overcome the blood brain barrier

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INTRODUCTION

The blood brain barrier is an important very tight barrier to restrict the penetration of substances into the brain. Brain capillary endothelial cells are linked together by tight junctions which are limiting the permeability (1) as well as the expression of efflux pumps that are part of an active defense mechanism (2). A lot of drugs such as antibiotics, virustatics and cytostatics reach only low concentrations at their site of action when administered parenterally and have to be applied directly into the brain blood vessels. MDCK (Madin-Darby canine kidney) cells are an accepted model to mimic the transport across the blood brain barier (3). To overcome this barrier, it was the aim of this study to investigate the potential of chitosans of different molecular masses in solution and as particles to enhance the transport into the brain.

EXPERIMENTAL METHODS

Preparation of chitosan nanoparticles

Chitosan was dissolved at pH 5.5 in a concentration of 0.15% (m/v). Thereafter, 0.2% (m/v) sodium triphosphate pentabasic (TPP) was added dropwise under permanent stirring until turbidity occurs. After 30 min the pH was risen to 6.4 for permeation studies and the particle size was determined by PSS Nicomp 380 ZLS particles sizer.

Table 1: Characterisation of the used chitosans

	Name	Company	Molecular mass	Viscosity [mPas]	Deacetylation degree
1	Short cut	Self prepared	20 kDa	Not detected	75-85%
2	Low-viscous	Fluka	150 kDa	<200	95-98%
3	Low molecular weight	Sigma	150 kDa	20	75-85%
4	Medium molecular weight	Sigma	400 kDa	200	75-85%
5	Highly-viscous	Fluka	600 kDa	>400	75-85%
6	High molecular weight	Sigma	600 kDa	800	>75%

Transport studies across MDCK monolayers

3x10^5 MDCK cells (passage no. 78-82) were seeded on polycarbonate Transwell membranes (mean pore diameter 0.45 μm) and maintained in DMEM (Dulbecco s modified Eagle s medium) with 10% FCS (fetal calf serum) at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. Cells grew and differentiated to confluent monolayers for 5-8 days. Permeation studies were performed in HBSS (Hanks balanced salts solution) buffered with 25mM HEPES (2-(4-(2-Hydroxyethyl)- 1-piper-azinyl)- ethansulfonsäure) with a pH of 6.4 at the apical and pH 7.4 at the basolateral site. The paracellular transport of 0.1% FITC-dextran MW 4kDa (FD4) was investigated in absence and presence of the different test compounds.

RESULTS AND DISCUSSION

The permeation of six different chitosans listed in Table 1 across MDCK monolayers was investigated. The results are shown in Fig. 1. Chitosans with molecular masses of 400 and 600 kDa increased the permeation of FD4 only 1.4 to 1.6-fold. The effect was much more pronounced by chitosans with molecular masses of 20 and 150 kDa (11.7 to 11.9-fold). These results are in good correlation with the results found by Shah et al. (4), who increased ^{99m}Tc-mannitol transport across MDCK cell monolayers 10.4-fold in presence of 0.3% of chitosan 20 kDa.

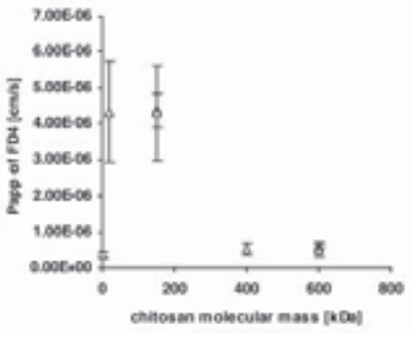

Figure 1: Correlation between the molecular mass of chitosan and the apparent permeability coefficents of FD4 across MDCK monolayers at pH 6.4. Indicated values are means (SD) of three experiments. All chitosan solutions were used in a final concentration of 0.25% (m/v) and differ from FD4 p<0.05.

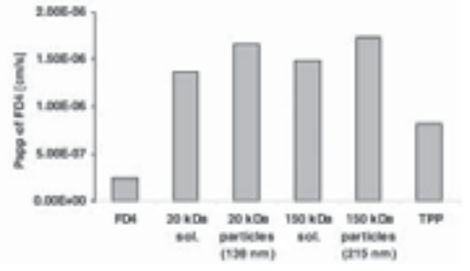

Figure 2: Comparison of the apparent permeability coefficients of FD4 across MDCK monolayers in presence of indicated test compounds in a final concentration of 0.1% (m/v) at pH 6.4. Indicated values are means of three experiments.

Chitosan particles also have an permeation enhancing effect, which is even 1.2-fold higher than the effect of the corresponding chitosan solution (Fig. 2).

CONCLUSION

The rank order for chitosan for paracellular permeation enhancement across MDCK cells is 20 kDa = 150 kDa > 400 kDa = 600 kDa. Furthermore particles formed by chitosan and TPP enhanced the transport 1.2-fold compared with chitosan solutions. Therefore 20 kDa chitosan and 150 kDa chitosan particles are useful ingredients for poorly absorbed drugs with the brain as site of action.

ACKNOLEDGEMENTS

The Austrian Nano-Initiative co-financed this work as part of the Nano-Health project (no. 0200), the sub-project NANO-N-0204 being financed by the Austrian FWF (Fonds zur Förderung der Wissenschaftlichen Forschung) (Project no. N-0204-NAN).

References

1. Schiera,G., Sala,S., Gallo, A., Raffa, M.P., Pitarresi, G.L., Savettieri, G., Di Liegro, I., 2005. Permeability properties of a three-cell type in vitro model of blood-brain barrier. Journal of Cellular and Molecular Medicine, 9, 373-379.
2. Löscher,W., Potschka, H., 2005. Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. Progress in Neurobiology, 76, 22-76.
3. Garberg, P., Ball, M., Borg, N., Cecchelli, R., Fenart, L., Hurst, R.D., Lindmark, T., Mabondzo, A., Nilsson, J.E., Raub, T.J., Stanimirovic, D., Terasaki, T., Oberg, J.O., Osterberg, T., 2005. In vitro models for the blood-brain barrier. Toxicology in Vitro, 19, 299-334.
4. Shah, P., Jogani, V., Mishra, P., Mishra, A.K., Bagchi, T., Misra, A., 2007. Modulation of ganciclovir intestinal absorption in presence of absorption enhancers. Pharmaceutical Technology, 96, 2710-22

OP005

Design of nanoparticles composed of poly (benzyl glutamate) derivates for targeted drug delivery to bone

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INTRODUCTION

Bisphosphonates are known to have strong affinities for hydroxyapatite (HA), a major inorganic component of hard tissues such as bone and teeth. The advantages of a bone-targeted drug delivery system for the treatment of bone diseases are obvious. Such a system could easily impart osteotropicity to a variety of bone drugs and improve their therapeutic potential. Skeletally targeted therapies have significiant opportunity in the areas of osteopororsis prevention, cartilage repair, cancer treatment, fracture repair and tissue engineering [1].

Poly (benzyl glutamate) (PBLG), a synthetic polypeptide, has attracted attention for biomedical applications because of the presence of a degradable amide bound in the polymer backbone [2]. To avoid the reticuloendothelial system (RES), a hydrophilic surface using PEG and small particle size are the most often mentioned criteria [3].

In this article, alendronate (ALD), a type of bisphosphonate, was chosen as the targeting moiety. PBLG copolymers were synthesized by ring-opening polymerization of γ-benzyl-L-glutamate N-carboxyanhydride (NCA) using selected amine-terminated initiators. The resulting polymers were analyzed using viscosimetry, Fourier Transform Infrared (FT-IR) and Nuclear Magnetic Resonance (NMR) spectroscopies. Polymeric bone-targeting drug delivery systems based on PBLG, PEG and ALD were designed. Nanoparticles smaller than 100 nm in diameter could be easily prepared from these PBLG derivatives by nanoprecipitation technique [4].

EXPERIMENTAL METHODS

• Synthesis and characterization of the PBLG derivatives

PBLG-PEG and PBLG-ALD were obtained by polymerization of NCA initiated by methoxy-PEG (MW=5000 Da) and ALD in dimethylformamide (DMF), respectively. The reactions were conducted under argon atmosphere and mixtures were stirred at 30°C until the characteristic FT-IR NCA bands disappeared from spectrum (Perkin-Elmer 1750). Further the mixture was precipitated in an excess of cold diethyl ether. The precipitates were filtered, washed with diethyl ether and finally dried under vacuum at 35°C. PBLG-Benzylamine was synthesized by the same method for PBLG-PEG-ALD polymerization. α,ω-Di-succinimidyl ester poly(ethylene glycol) (MW_{PEG} = 6000 Dalton) was utilized as a linker to incorporation of ALD to polymer.

To determine the average polymer molecular weights using the Mark–Houwink equation *($[\eta] = 1.58 \times 10\text{-}5Mw^{1.35}$)*, intrinsic viscosity (η) was measured in DMF at 25 °C using an Ubbelohde viscometer. ^{1}H-NMR and ^{31}P-NMR spectras were recorded in deuterated chloroform ($CDCl_3$) with a Bruker Advance 400 and 200 apparatus, respectively.

• Nanoparticles preparation and characterization

Nanoparticles from PBLG-Bnz, PBLG-PEG, PBLG-ALD, PBLG-PEG-ALD and commercially available PBLG were prepared by a nanoprecipitation method. Briefly, 15mg of polymer was dissolved in 5mL of THF at 30 °C. This solution was added by dripping to 10mL of milli-Q water under magnetic stirring. Then the mixture was transferred in a

Teflon surface. The solvent was evaporated, at 30 °C, under a light air flow. Nanoparticles were washed with 5mL of milli-Q water and evaporation was carried out to yield 10mL of nanoparticles suspension. The mean diameter (n=3) was determined by dynamic laser light scattering (Nanosizer Coulter N4 Plus, Margency, France) and also by their observation in transmission electron microscopy (TEM) (Philips EM 208). The surface-modified nanoparticles with PEG and ALD were evaluated by adsorption study onto HA.

RESULTS AND DISCUSSION

Polymerizations were followed by FT-IR spectroscopy. Disappearance of absorption bands for the cyclic 5-ring anhydrides at ~1850, ~1775 and ~920cm^{-1} indicated the end of the polymerization reaction.
Molecular weight of PBLG derivatives (Table 1) were determined by viscosity measurements and ^{1}H-NMR.

Table 1: Molecular weights of synthesized polymers

Polymer	MWt (gmol^{-1})	Reaction time	Yield (%)	MWp (gmol^{-1})
PBLG-Bnz	50 000	4 days	97	45 000
PBLG-PEG	60 000	8 days	97	55 000
PBLG-ALD	50 000	15 days	98	48 000
PBLG-PEG-ALD	60 000	6 days	99	70 000

The degree of polymerization, DPn, of PBLG segments in PBLG-PEG derivatives were determined from ^{1}H NMR spectra by the ratio between the peak intensities of methylene protons of the PEG chain, OCH_2CH_2, and the benzyl protons of PBLG chain, $COOCH_2C_6H_5$. Values of DPn of 274 were found for the PBLG-PEG and PBLG-PEG-ALD (Fig. 1a). For the other PBLG derivatives, DPn measurements were not possible by ^{1}H NMR because the initiator signal was difficult to localize due to the presence of other peaks in the same region. ^{31}P-NMR = 20.42 and 19.81 ppm for PBLG-ALD and PBLG-PEG-ALD polymers, respectively (Fig. 1b).

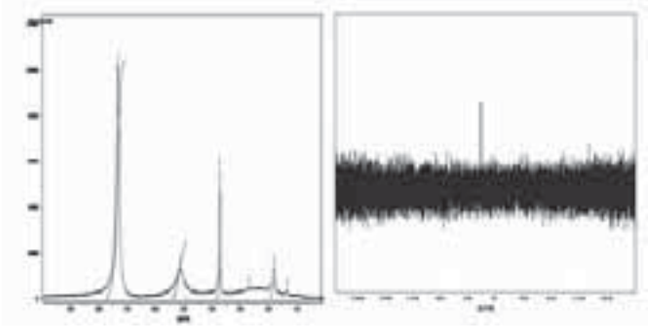

Figure 1: a) ^{1}H-NMR spectra of PBLG-PEG-ALD
b) ^{31}P-NMR spectra of PBLG-ALD

• Nanoparticle Characterizations

Nanoparticles could be easily and reproducibly obtained from the different polymers. Their diameters were typically less than 100 nm and narrow size distribution were obtained, depending on the polymer (Table 2), which was confirmed by TEM (Fig. 2). The stronger potential value was found for non-PEGylated nanoparticles (Table 2).

Table 2: Characteristics of PBLG derivative nanoparticles

Polymer	Mean Size (nm)	Polydispersity index	ζ potential (mV)
PBLG-com	64 ± 15	0.108	-40.2 ± 0.88
PBLG-Bnz	78 ± 11	0.119	- 30.7 ± 0.35
PBLG-PEG	44 ± 20	0.258	-15.5 ± 0.31
PBLG-ALD	59 ± 21	0.132	-40.3 ± 0.86
PBLG-PEG-ALD	66 ± 24	0.128	-34.5 ± 0.80

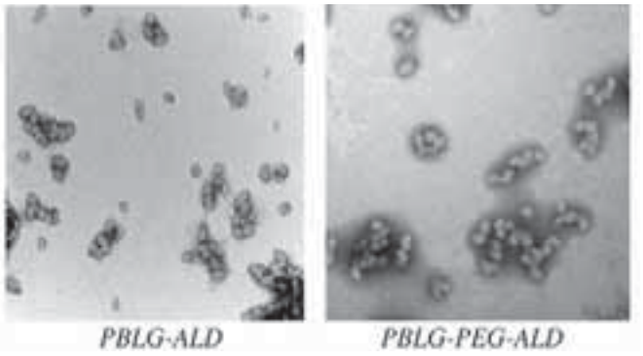

Figure 2: TEM micrographs of bone targeted nanoparticles

CONCLUSION

Degradable PBLG derivatives were synthesized using an easy and reproducible polimerization method. HA affinity assay suggested that functional nanoparticles were designed by surface modifications with ALD (targeting moiety) and PEG (hydrophilicity). Small nanoparticles could be prepared by nanoprecipitation technique. These nanoparticles may be used as universal vehicles for the targeted delivery of bone therapeutics.

References

1. S.W. Choi, J.H. Kim, J. Control. Release **122** 24–30 (2007)
2. E.M. Barbosa, V. Montembault, S. Cammas-Marion, G. Ponchel and L. Fontaine, Polym Int **56** 317–324 (2007)
3. Y.I. Jeong, S.J. Seo, I.K. Park, H.C. Lee, I.C. Kang, T. Akaike, C.S. Cho, Int. J. Pharm. **296** 151-161 (2005)
4. Thioune O, Fessi H, Devissaguet JP and Puisieux F, Int. J. Pharm. **146** 233 (1997)

OP006

Peptide-based cationic liposomes and micelles as a new tool for nonviral gene delivery

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INTRODUCTION

Non-viral systems, such as cationic lipids, peptides, glycopeptides, liposomes, micelles, glycosilated polymers, micro- and nanoparticles, possess in general low efficiency and transient expression with respect to viral systems, but also very low potential toxicity (1). In the present study the use of new synthesised cationic peptides (CPs) as cationic lipids for the production of non-viral gene delivery systems, such as cationic liposomes and micelles was considered. Particularly, the cell toxicity and the ability of these peptides-based cationic systems to bind DNA were investigated. As model DNA, Defibrotide (DFT) a single strand polydeoxyribonucleotide sodium salt extracted from mammalian organs (mean molecular weight comprised between 15,000 and 30,000 daltons) was used (2).

METHODS

Five CPs, namely tetralysine cholesteryl hemisuccinate, tetralysine palmitate, tetralysine 3,7-diacetyl-ursodeoxycholate, tetralysine L-aspartic acid β-phosphatidyl-ethanolamine and tetralysine S-farnesyl-L-cysteine (see Figure 1) were prepared by solid-phase method then isolated, purified and analytically characterized. CP-liposomes were prepared by REV-phase and extrusion (3). The molar ratio of the constituents was phosphatidylcholine:CP 4:1 (mol/mol).

CP-micelles were obtained by simple addition of different amounts of CPs in sterile water. The complexes were obtained by mixing CP-micelles or CP-liposomes with an aqueous solution of DFT.

Liposomes and micelles were characterized by size and charge using a Zetasizer 3000, Malvern Instr., UK.

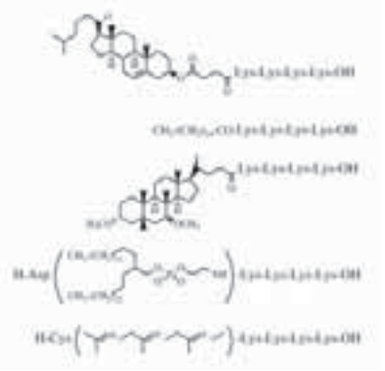
Lipopeptide	Nickname
	LCS
	LP
	LUDC
	LAPE
	LFaC

Figure 1: Chemical structure and nicknames of cationic peptide molecules

Liposome/DFT complexes were incubated at 37°C for 5 min, and then each sample was subjected to electrophoresis. Electrophoresis was performed in 3% agarose gel at constant voltage (25 mV) for 2 hours, in the absence or in the presence of rehydrated liposomes. The relative band migration was determined, after staining the gels with ethidium bromide.

The effect of the different cationic formulations was determined on in vitro cultured human K562 erythroleukemic cells.

RESULTS

The plain CP-liposomes present an average diameter reflecting the pore size of the membrane used for the extrusion (i.e. 100 nm, data not shown). After DFT complexation the mean diameter of complexes decreased by increasing the positive charges number (Figure 2). The non-complexed liposome preparations showed a net positive zeta potential comprised between + 17.8 and + 30 mV. After adding DFT to liposomes (at a 1:4 +/- molar ratio) the zeta potential fell down to a net negative value indicating the formation of the ionic complex. Concerning micelles, before complexation it was not possible to measure their size by PCS. However, after DFT complexation the size of complexes highly increased (Table 2).

Table 2: Mean diameter by intensity of CP-based micelles after complexation with DFT, as determined by PCS

CP	+/- molar charge ratio				
	1:4	1:1	2:1	4:1	8:1
LCS	266 ± 24	205 ± 19	180 ± 26	153 ± 23	141 ± 15
LP	347 ± 27	339 ± 22	333 ± 31	253 ± 28	213 ± 21
LUDC	280 ± 19	216 ± 19	157 ± 24	146 ± 15	130 ± 12
LAPE	578 ± 32	416 ± 36	239 ± 33	200 ± 26	170 ± 17
LCFa	184 ± 15	154 ± 11	115 ± 18	109 ± 12	97 ± 11

In addition, before complexation, the five CPs solutions showed a positive zeta potential ranging from +10 to +17.8 mV, while after addition of DFT the zeta potential fells to negative values.

The ability of CP-liposomes and CP-micelles to complex DNA was studied by mean of agarose gel electrophoreses (3). The reduction of the electrophoretic migration band intensity of DFT was considered as indication of the formation of a complex. In this view, LCS showed a complete DFT association at +/- molar charge ratio (+/- m.c.r.) comprised between 4:1 and 8:1 both for liposomes and micelles. The band of free DFT disappears at +/- m.c.r. comprised between 2:1 and 4:1 for LP-li-

posomes and between 8:1 and 16:1 for LP-micelles. Concerning LUDC-liposomes, the band of free DFT disappeared starting from 4:1 and finishing at 16:1 +/- m.c.r. while LUDC-micelles complex DFT at a +/- m.c.r. comprised between 2:1 and 4:1. Both LAPE liposomes and micelles showed a complete disappearance of the DNA migration band at a +/- m.c.r. comprised between 8:1 and 16:1.

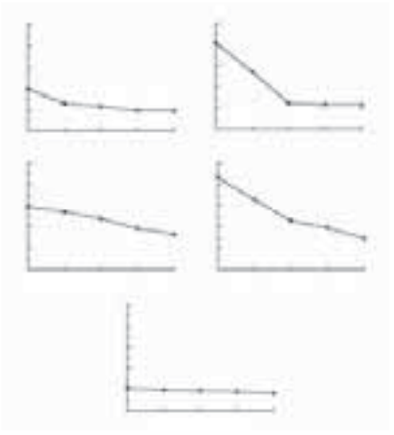


Figure 2: Effect of complexation of DFT at different +/- charge molar ratio (namely 1:4, 1:1, 2:1 and 8:1 mol/mol) on size of 100 nm extruded CP-liposomes. A: LCS. B: LP. C: LUDC. D: LAPE and E: LCFa.

Among the five formulations, the lower ability in complexing DNA was shown by LCFa-liposomes, whose migration band disappeared at 16:1 +/- m.c.r. In the range of +/- m.c.r. used, LCFa-micelles do not evidenced a band disappearance.

In order to obtain information about the toxicity of CP-based molecules, human erythroleukemic K562 cells were cultured in the presence of CP-formulations. Concentrations ranging from 0 and 50 μM were tested. The results reported in Figure 3 indicate that except in the case of LCFa, liposomes displayed a lower toxicity towards K562 cells compared to that of the corresponding micelles. Particularly, LC-based formulations showed an IC_{50} value of 12.3 μM for liposomes and 23.1 μM for micelles. The substitution of cholesterol with palmitic acid led to an increase in toxicity of both the formulations, moving the IC_{50} values around 6 μM.

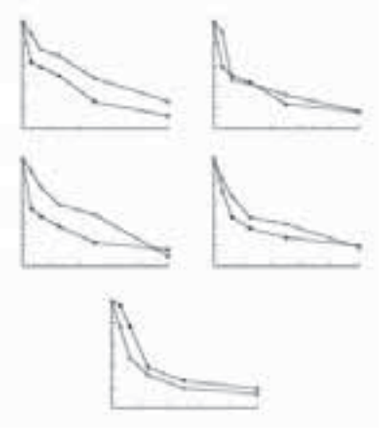


Figure 3: Effect of CP-liposomes (filled symbols) and CP-micelles (open symbols) on K562 cells growth. A: LCS. B: LP. C: LUDC. D: LAPE and E: LCFa.

CONCLUSIONS

Taking into account these results, the studied CPs could be efficiently used to obtain both cationic liposomes and micelles. Moreover they are able to complex DNA with different interaction strength, depending on the type of peptide-based cationic molecule used.

References

1. Pietersz G.A. et al. Structure and design of polycationic carriers for gene delivery. Mini Review Medicinal Chemistry, 6, 1285-98 (2006).
2. Cimminiello C. Clinical trials with defibrotide in vascular disorders. Semin. Thromb. Hemost., 22, 29-34 (1996)
3. Cortesi R. et al. Effect of cationic liposome composition on their in vitro citotoxicity and protective effect on carried DNA Int. J. Pharm. 139, 69-78 (1996).

OP007

CFD simulation of two-phase flow in Wurster chamber and its experimental validation

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INTRODUCTION

Coating of pharmaceutical products is a common process with which one can achieve protection of the active pharmaceutical ingredient and modified or controlled release. Coatings are usually polymer based and can include other excipients such as plasticizers, colorants [1].

Coating of pellets is usually done in fluid bed device such as Wurster chamber. Wurster chamber is a bottom spray device and it's important element is a draft tube where the coating takes place. As any other fluid bed device also Wurster chamber has many process parameters, which are subject of optimization. More important ones include product load-

ing, mass flow of fluidizing air, spraying nozzle position, atomizing air flow rate and the position of the draft tube [2].
Computational fluid dynamics (CFD) use numerical methods and physical models to solve and analyze different problems that involve fluid flow. With CFD modeling we can optimize different process parameters, increase our understanding of the process and get information about different process values that we otherwise could not measure or are hard to measure [3].
The aim of our study was to analyze the volume fraction of pellets inside the draft tube using CFD analysis and to validate these results using experimental methods.

METHODS

Equipment and materials

- Wurster chamber GPCG 1 (Glatt GmbH, Germany) with custom hatch to close the top of the draft tube was used in all experiments
- Modified hover system (Rowenta) was used to retrieve pellets from the chamber after stopping the system in order to determine the mass of pellets within and directly below the Wurster draft tube
- 1085 g (equivalent of 1190 cm^3) of sugar pellets with average diameter of 1.013 mm were used.
- CFD computation and post processing was done using Fluent 6.3.26, model design and computational grid generation was done using Gambit 2.3.16 (Figure 1)

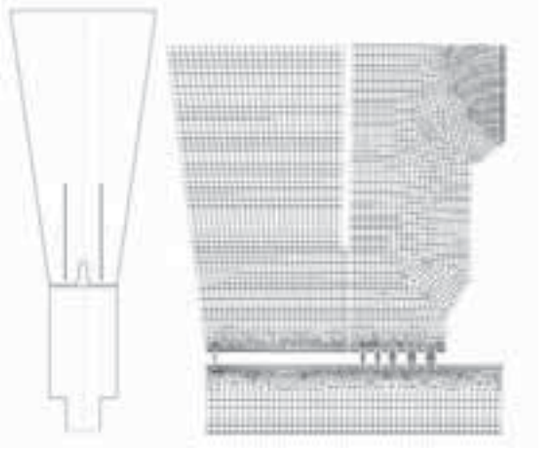

Figure 1: 2D Wurster geometry (left) and detail of computational grid in area of distribution plate, draft tube and spraying nozzle (right).

Procedures

- Measurements were performed at three different fluidizing air flow rate: 1.82, 2.52, 3.17 kg/min and at the different Wurster gap settings: 10, 20 and 25 mm resulting in 8 measurements; simulations using 2D axisymmetric model with approximately 30,000 computational cells were done for each measurement
- Atomizing air pressure was set at 2 bar in all experiments and no coating suspension was applied during the experiment, nozzle air inlet in simulation was defined according to previous measurements
- Eulerian two phase model was used for unsteady CFD simulation of air and pellets in the system, where Gidaspow drag model was used and each simulation was performed for at least 5 seconds.

RESULTS

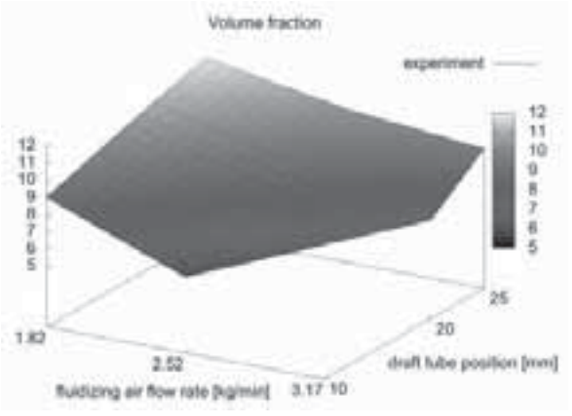

Figure 2: Volume fraction of pellets inside the Wurster draft determined experimentaly.

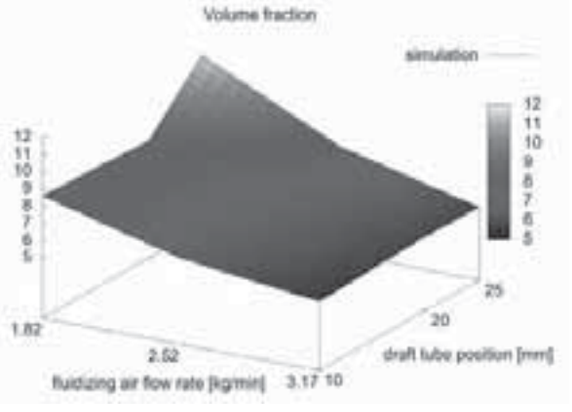

Figure 3: Volume fraction of pellets inside the Wurster draft determined by the simulation.

DISCUSSION

From figure 2 and 3 we can observe differences in simulated and experimental values of the volume fraction of pellets inside and below the Wurster draft tube. First thing one can observe is that the simulated values are generally lower than experimental ones, which can be explained by the experimental measuring method for the volume fraction. During the experiment the GPCG 1 was firstly turned off and then pellets inside and below the draft were separated and afterwards weighted. During the time when the device was turned off, some of the solid material might migrate below the draft tube and virtually increase the value of volume fraction.
One value from simulation clearly deviates from the rest – that is the value of volume fraction from simulation that was done at air flow rate of 1.87 kg/min and Wurster gap of 25 mm. Air speed in this case is the lowest tested, whereas the Wurster gap is at the highest position and from CFD solution animation one can observe different flow regime of the pellets.
Based on experimental results and the results of simulation we can conclude that increasing inlet air speed, lowers volume fraction of pellets

within the draft tube, which can have significant effect on quality or variance of coating. The effect of Wurster gap is not so pronounced in simulation as in experiment. It is assumed that this is due to the CFD code limitation to model dense flow of solids in horizontal transport region of Wurster chamber.
Observation of simulated flow patterns such as e.g. movement of pellets near the walls of draft tube is similar to observations in reality. Also some preliminary CFD readings of pellets' velocity have shown good agreement with experimental determination of pellets' velocity.

CONCLUSION
Although the results of simulation are not entirely correct, we can use this simulation to further optimize coating processes and study various process parameters such as loading, density of material and others.

References

1. Y. Fukomori, H. Ichikawa *Encyclopedia of Pharmaceutical Technology* Informa Healthcare 1773 – 1779 (2006)
2. M. Aulton, *Pharmaceutics* Elsevier, 372 – 386 (2002)
3. J. Wendt *Computational Fluid Dynamics: An introduction*, Springer 1996

OP008

Innovative fluid bed pelletising technologies for matrixpellets and micropellets

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INTRODUCTION
In multi-particulate systems the dosage of a drug substance is – in contrast to classic single-unit dosage forms like tablets – divided on a plurality of sub-units, consisting of pellet particles with a diameter of typically 100 – 2000 μm.

Although their manufacture and design is more complex in comparison to classic single-unit dosage forms, multi-particulate formulations offer a magnitude of interesting options and advantages to accomplish unique product characteristics and in particular specific drug release patterns.

With multiparticulate pharmaceutical drug applications an optimised pharmacokinetic profile can go together with good patient compliance. Many creative formulation options ending up with intelligent, sophisticated and reliably acting pharmaceutical dosage forms are basically available. The question is: do we have feasible processing technologies in order to establish reproducible product and process quality?

Pellets can be formulated to different drug applications: capsules, tablets, sachets, oral liquids and ODT (orally disintegrating tablets). Especially when taste masked micropellets are the objective particular pelletising technologies have to be applied. With classic fluid bed drug layering and coating technologies like the Wurster and the Rotor technology pellet particle sizes < 300 – 500 μm are basically achievable taking into account that the Wurster process is limited to drug layering approaches. An optimised Rotor technology could lead to an even better performance than the existing one.

In addition to said existing and established pelletising technologies innovative technologies allowing new formulation options and product qualities are presented. In particular, unique benefits and opportunities such as a small pellet size range of 100 – 500 μm, uniformity of particle size distribution, smooth particle surface, high density and high drug loading are achievable.

EXPERIMENTAL METHODS
- **CPS™ Technology**

Glatt GPCG 1 with CPS™ 3 configuration for labscale batch size; Glatt GPCG 30 with CPS™ 30 configuration for pilot scale batch size; as the results were achieved from commercial development projects with industrial partners the API's processed cannot be disclosed.

- **MicroPx™ Technology**

Glatt Pharmalab unit for lab scle trials; Glatt GPCG 30 / 60 with MicroPx™ configuration for pilot scale application; Glatt GPCG 120 with MicroPx™ configuration for commercial production; as the results were achieved from commercial development projects with industrial partners the API's processed cannot be disclosed.

- **ProCell™ Technology**

Glatt ProCell™ 5 unit for lab scale trials; Glatt ProCell™ 70 unit for pilot scale trials;

RESULTS AND DISCUSSION
- **CPS™ Technology (fig. 1)**

The **C**ontrolled Release **P**elletising **S**ystem (CPS™) Technology is an advanced fluid bed rotor technology allowing the preparation of matrix pellets with particular properties in a direct pelletisation batch process; extremely low dosed and high potent API's can be formulated to matrix pellets as well as high dosed APIs; the drug concentration can vary from < 1% up to 90%.
Inert starting beads are not required; together with the frequently used microcrystalline cellulose powder functional excipients like polymers, disintegrants, solubilizers and the like can be part of the CPS™ formu-

lations in combination with the API. Applying a defined set of processing paramters incl pelletisation forces throughout the process a controlled release of drug from the matrix pellets is achieved.

- **MicroPx™ Technology (fig. 2)**

The MicroPx™ Technology provides matrix type micropellets with a particle size < 300 - 400 µm and a drug load of typically ≥ 95%. Functional pharmaceutical excipients, e.g. for bioavailability enhancement or controlled drug delivery can be integrated in the pellet matrix.
The formulation components are processed with a continuous fluid bed process for which no starting cores are required. Typically, all components like the API, pharmaceutical binder(s) and functional ingredients are contained in a liquid - solution, suspension, emulsion or the like - which is fed into the process via spray guns. Particle size is controlled with the help of an online classification system. The MicroPx™ technology is ideally be applied when taste-masked micropellets for the use in oral suspensions, sachets or ODT forms must be provided.

- **ProCell™ Technology (fig. 3)**

The ProCell™ Technology is a spouted-bed type pelletising process for the preparation of very high concentrated particles; ideally, no additional excipients may be required.

For the direct granulation and pelletising process no inert starting beads are required and either, solutions, suspensions, emulsions be processed. The most effect process is achieved when a melt of e.g. pure API is processed, as in this case neither solvents nor excipients are required.
With Ibuprofen as a model drug a 100% direct compressable granule can be manufactured.

CONCLUSION (fig. 4)

The innovative fluid bed pelletising technologies complement the actual fluid bed pelletising capabilities: in addition to established pelletising approaches new additional options are made available.
A variability of product qualities related with different product throughputs and manufacturing cost are realizable. In particular micropellets being an ideal substrate for taste masked particles for oral suspensions, sachet or ODTs are provided.

References

1. Pöllinger, N.; Glatt International Times **25**, 2 – 7 (2008)
2. Schlütermann, B.; 7th Syntapharm Workshop, Berlin May 10 – 11 (2007)

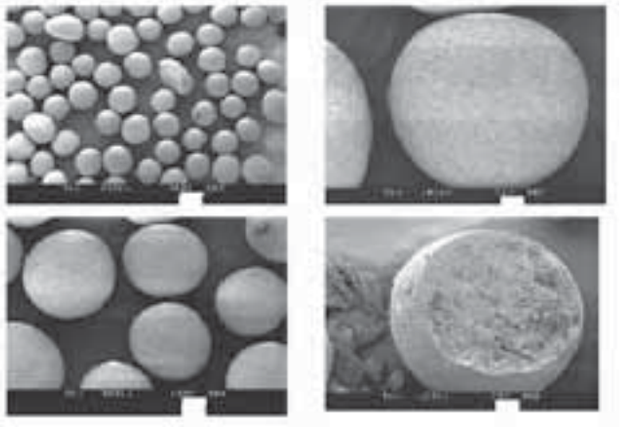

Figure 1: CPS™ Matrix Pellets

Figure 2: MicroPx™ Technology

Figure 3: ProCell™ Technology

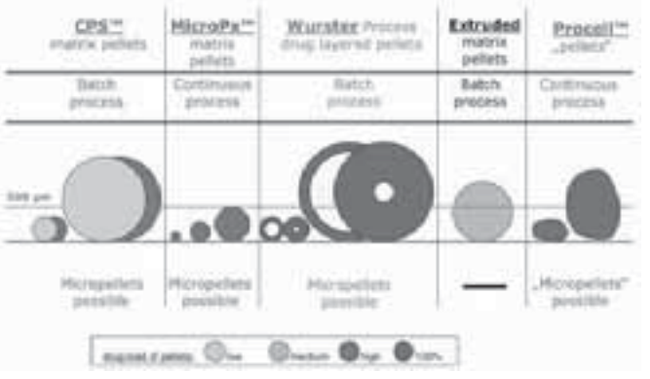

Figure 4: Product Characteristics with different pelletisation technologies

OP009

Near infrared spectroscopy (NIR) as a method for tablet crushing strength determination

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INTRODUCTION

NIR (Near Infrared Spectroscopy) is a powerful analytical technique with a wide range of applications in the areas of drug discovery, product development and manufacturing in pharmaceutical industry. It is rapid and non-destructive analytical method which can simultaneously gain analytical and physical information about the sample which ensure that it becomes an important tool in the current PAT (Process Analytical Technology) initiative [1,2].

NIR has been successfully used for material identification, determining endpoint during powder mixing, moisture determination, tablet assay and crushing strength determination, detection of different polymorphic forms of same compound, particle size determination because NIR spectra are influenced by different physical properties of material such us particle size and shape [3].

The mechanical strength of tablet plays an important role in the total quality of the product. It is usually determined during the tableting as tablet crushing strength test which bases on determination of compression force which just broke tablet when applied diametrically on it. This technique has some limitations such as: it is often a subject to operator error, all tablets can not be analysed while it is a destructive method, the corresponding settlement of tableting press parameters can be performed only with certain time-lag, during which a certain quantity of tablets which potentially does not fit the tablet hardness specifications can be produced. As an alternative NIR technique can be used as on-line, non-destructive technique where all tablets can be tested. It has been demonstrated that using NIR on tablets, signal varies according to a change in tablet hardness or using different compression pressures during tableting. This is a base for creating a model which can be used for predicting tablet hardness [4].

EXPERIMENTAL METHODS

NIR model was developed based on analysing tablets which were made using experimental design plan (design space) including different combinations of process parameters on tableting machine: main compression pressure, precompression and tableting speed. The formulation includes 75% of active ingredient (macrolide antibiotic). Three types of tableting mixtures with the same qualitative and quantitative composition have been made: a) mixture for direct tableting (DIRECT) and two types of dry granulated mixtures: b) compacted on roller compactor (COMPACT) and c) slugged on rotary tablet press (BRIKET). Tableting was performed on industrial rotary tablet press Killian T300/40 (IMA, Germany) using concave shape punches /Φ=13 mm; R= 26 mm/.

Each sample was first scanned in transmission mode at wavelength 12000-7000 cm^{-1} (Bruker MPA NIR), afterwards tablet hardness was determined by using standard Erweka TBH 300MD tablet hardness tester. Using linear regression method predictive model was created. Principal Component Analysis (PCA) was used to evaluate potential effect of structural changes during compression (amorphisation) on NIR spectre.

RESULTS AND DISCUSSION

Tablets which were compressed at higher compression pressure have higher tablet hardness, higher tablet density and lower porosity which lead into corresponding lower signal on a detector (InGaAs detector – transmission mode) and parallel, lower absorption intensity through the entire NIR spectrum (Fig.1).

Based on NIR absorbance and classical diametral hardness test results, a quantitative calibration model was developed which included the results of all three tested systems (DIRECT, COMPACT and BRIKET). The correlation coefficient R^2= 88,89 (Fig.2).

Principal component analysis (PCA) of the NIR spectra indicate that the first and second principal components correlated with the type of powder for tableting (direct tableting or dry granulation) (Fig.3). There is also minor difference between spectres of tablets made from roller compacted and slugged systems. Beside the difference in particle size it was confirmed in following studies that dry granulated systems have significant higher level of particle amorphisation than direct tableting systems [5] which affect also NIR spectres. However, calibration models created for each system separately didn't have higher R^2 values,

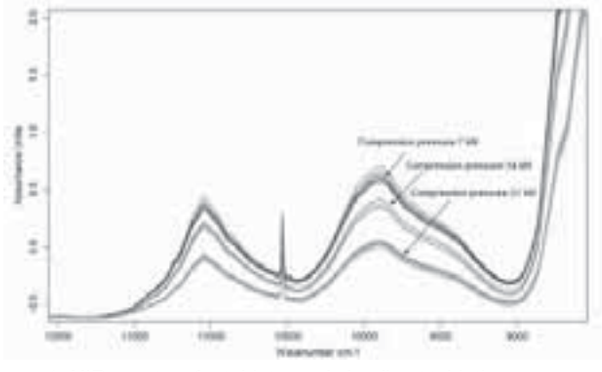

Figure 1: NIR spectres for tablets made by direct tableting compressed at different compression pressures.

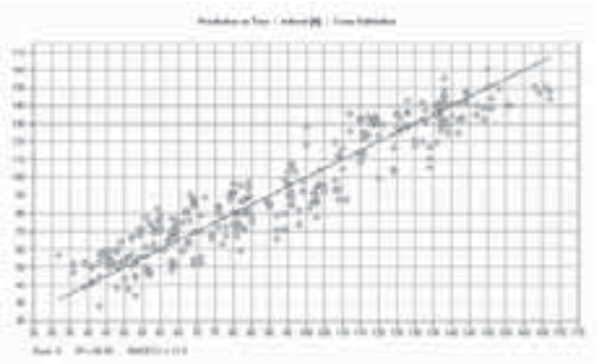

Figure 2: Model for predicting tablet hardness for DIRECT, COMPACT and BRIKET.

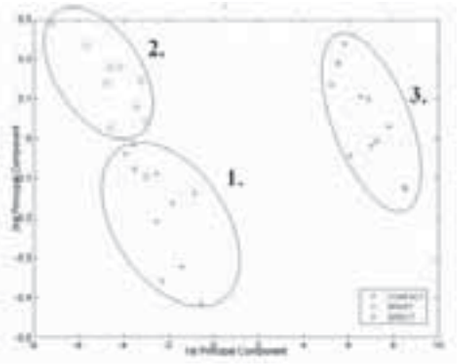

Figure 3: PCA analysis of NIR spectres for tablets DIRECT, COMPACT and BRIKET.

probably because of smaller number of samples included in each model.

CONCLUSION

NIR can be used effectively for the non-destructive prediction of tablet crushing strength as an alternative to a classical diametral hardness test for tablets prepared by direct tableting or using dry granulation process. The absorbance spectra is influenced by particle size and structural changes of particles (amorphisation), however the model which include both techniques have relative high prediction power.

References

1. FDA: PAT initiative: http://www.fda.gov/cder/OPS/ PAT.htm
2. Cogdill RP, Anderson CA, Drennen JK. Using NIR Spectroscopy as an integrated PAT tool. Spectroscopy; 19 (12): 104–109 (2004).
3. Reich G. Near-infrared spectroscopy and imaging: Basic principles and pharmaceutical applications. Advanced Drug Del Rew; 57: 1109–1143 (2005).
4. Roberts RA, Kemper MS, Rubinovitz R, Martin DL, Reier GE, Wheatley TA, Shukla AJ. Application of near-infrared spectroscopy for nondestructive analysis of Avicel powders and tablets. Pharm Dev Tech; 4(1): 19–26 (1999).
5. Zupancic Bozic D, Dreu R, Vrecer F. Influence of dry granulation on compactibility and capping tendency of macrolide antibiotic formulation. Int J Pharm; 357: 44-54 (2008).

OP010

Preparation of ascorbic acid containing chewing gum tablet by direct compression

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INTRODUCTION

The dosage form or delivery system is critical to the success of a pharmaceutical product. Today chewing gum tablet is gaining new consideration as drug delivery system; it provides additional patient benefits and compliance, new competitive advantages from technological and marketing view point. Medicated chewing gum tablets are solid, single-dose preparations that have to be chewed and not swallowed; chewing gum tablets contain one or more active ingredients that are released by chewing.

Generally the chewing gum tablets consist of a different neutral and tasteless gum-base and several other ingredients, such as fillers, softeners, sweeteners, flavoring and texture regulating agents.

EXPERIMENTAL

• Materials

Preparing the chewing gum tablets different gum bases, Pharmagum™ C, M and S (SPI Pharma, New Castle, DE), ascorbic acid (Ph. Eur.) as a model drug, xilitol (Roquette, Lestrem, France) as sweetener, orange aroma, and magnesium stearate as lubricant (Ph. Eur.) were used.

• Methods

Preparing the powder mixture a Turbula mixer was used. Investigating the flowing properties of the raw materials and the powder mixtures a Pharmatest PTG-1 powder tester was applied. Checking the compactibility and the density of the materials an Engelsmann Stampfvolumeter was used. The true density was investigated with a Quantachrome pycnometer. The chewing gum tablets were compressed with a Korsch EK0 instrumented eccentric tablet machine with 10 mm in diameter flat and beveled punches, with three different compression forces 5, 10, and 15 kN. The friability investigation of the chewing gum tablets was performed with an Erweka friabilator. For checking the breaking hardness of the chewing gum tablets a self developed hardness tester was used.

RESULTS

Checking the tabletting properties of the different gum bases six different compositions were prepared (table 1.)

The amount of the other materials in all of the compositions was the next (table 2.).

From the three different gum bases only the Pharmagum S shows flowing properties. Checking the powder mixtures all of the compositions have a good flow property but the Sample 4-5-6 has a shorter flowing time than the Sample 1-2-3 (table 3.).

Table 1: Composition of gum base mixtures used for preparing the chewing gum tablets

	Pharmagum™ (%)		
	C	M	S
Sample 1	60	40	-
Sample 2	50	50	-
Sample 3	40	60	-
Sample 4	60	-	40
Sample 5	50	-	50
Sample 6	40	-	60

Table 2: Amount of the other materials in the chewing gum tablets

Material	Amount (%)
Ascorbic acid	8.33
Xilitol	7.68
Aroma	0.33
Magnesium-stearate	2.00

Table 3: Flowing properties of the gum bases and mixtures

	Flowing time (s)	Angle of repose (°)	Heap volume (ml)	Heap mass (g)	Apparent density (g/ml)
Phg C	n.m.	n.m.	n.m.	n.m.	n.m.
Phg M	n.m.	n.m.	n.m.	n.m.	n.m.
Phg S	7.5	26.2	64.4	56.6	0.878
Sample 1	12.3	29.3	73.4	58.8	0.801
Sample 2	10.3	29.4	73.9	59.7	0.808
Sample 3	10.0	29.8	75.0	58.8	0.784
Sample 4	7.6	26.6	65.6	66.9	1.020
Sample 5	7.3	27.5	68.2	67.8	0.995
Sample 6	7.4	28.3	70.6	65.5	0.929

n.m. = not measurable

During tablet making it was founded that the raw materials and the mixtures has a different sticking properties to the punches (table 4.). The result shows that the Pharmagum C and M has a high sticking property. There was no possibility to prepare a tablet from that gum bases only because the material sticked to the upper punch. The true density was investigated from the gum bases, from the powder mixtures, and the tablets. During compressing the powder mixtures Sample 1, 2, and 3 shows a sticking property also. In the case of Sample 4, 5, and 6 only the Sample 6 showed a sticking property after preparing a large amount of tablets. To avoid the sticking properties of the materials a self adhesive teflon film was applied on the surface of the upper and lower punch.

Table 4: Sticking properties of gum bases and powder mixtures

	Pharmagum			Sample					
	C	M	S	1	2	3	4	5	6
St	+	+	-	+	+	+	-	-	+/-

St = sticking properties

After preparing the tablets the breaking process was investigated. As the results shows there is no significant difference from the breaking hardness values (table 5.). It seems that the compression force did not determine the breaking hardness and process. The only difference is a breaking time but it is not increases significantly with the increase of the compression force. In comparison the breaking process of the chewing gum tablet to the traditional preparation it seems that the process is not breaks down immediately (fig. 1). The tablet did not break into two parts (or more) because of the elasticity.

Table 5: Breaking properties of chewing gum tablets

	PF (kN)	BT (ms)	BF (N)		PF (kN)	BT (ms)	BF (N)
S 1	5	1936	6.00	S 4	5	1564	6.28
	10	2148	6.80		10	1764	7.41
	15	2228	7.22		15	1676	7.39
S 2	5	2008	6.66	S 5	5	1532	6.34
	10	2108	7.22		10	1600	7.35
	15	2192	7.47		15	1736	7.20
S 3	5	2056	7.60	S 6	5	1808	5.99
	10	2184	6.84		10	1804	6.85
	15	2152	7.12		15	1860	7.32

PF= pressure force during tablet making; BT= breaking time; BF= breaking force

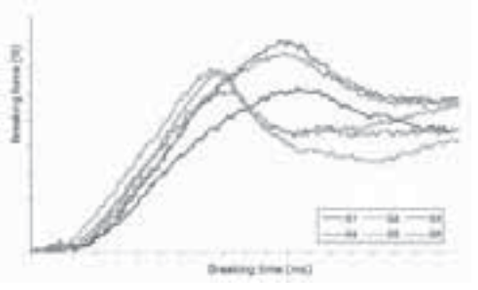

Figure 1: Breaking process of chewing gum tablets prepared with 5 kN pressure force

Conclusions

From the different gum bases there were difficult to prepare a chewing gum tablets with a conventional punches because of the sticking properties of the materials. It was necessary to cover the punch surface with a teflon film. The breaking property of the tablets is more different from the traditional tablets. The tablets show an elastic property in the post-compressional test. For suitable chewing this property is important in this type of dosage form.

Acknowledgement

The authors thank the gum base materials for Lehmann & Voss Co

OP011

Quantifying water uptake into controlled drug delivery polymers using ultra-fast MRI method

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INTRODUCTION

Controlled drug delivery systems are extensively used and researched by the pharmaceutical industry. Hydrophilic polymer matrices such as hydroxypropyl methylcellulose (HPMC) is one example of a formulation that is used for controlled drug release. When HPMC is exposed to aqueous liquids, the hydrated polymer starts to swell and forms a viscous mucilaginous gel layer, which forms a diffusion barrier to further water uptake and drug release. This gel layer is regarded as the controlling mechanism for drug release. The polymer hydration process is one of the key issues that is thought to influence the drug release kinetics (1). In this work, ultra-fast magnetic resonance imaging (MRI) techniques were developed to quantify water uptake by HPMC. This powerful non-invasive technique has been applied in various areas of research, including clinical medicine (2) and chemical engineering applications (3). Its potential use in the pharmaceutical industry has recently been reviewed with a particular focus on controlled drug release dosage forms (4). However, a major disadvantage of traditional spin-echo (5) MRI methods is that the time taken to obtain a single ***quantitative*** two-dimensional slice selective image is several hours. Therefore standard MRI techniques are not appropriate for examining 'fast' or 'medium' release controlled delivery systems where the timescales are of the order of a few hundred milliseconds to several hours. It is possible to reduce MR image acquisition times to tens of milliseconds by applying ultra fast imaging techniques, but these methods are in general non-quantitative. Here we report the development of the ultra fast RARE imaging method (6) to study the water/HPMC system. In particular, these new MRI methods give rapid non-invasive measurements of: (i) the dynamics of water uptake into the gel layer; (ii) the absolute concentration of water uptake in the gel layer; (iii) the self-diffusivity of water within the gel layer.

EXPERIMENTAL METHODS

All experiments were performed on a Bruker AV 400 NMR spectrometer equipped with micro imaging accessory, operating at a ^{1}H frequency of 400.225 MHz. A vertical birdcage 25 mm diameter proton coil was used. The disc shaped HPMC tablets (Dow Methocel K100LV) were 12 mm in diameter and 4 mm in thickness. Tablets were prepared by direct compression of HPMC powder in a Carver press. The tablet was then placed into a 25 mm plastic tube and was fixed into place by the use of Blu-Tack 10 ml of deionised water was then added to the tube. T_2-preconditioned and diffusion preconditioned RARE imaging methods were applied in succession to give quantitative water concentration, and water self-diffusion maps respectively. Complete sets of T_2-preconditioned (9 images) and diffusion preconditioned (8 images) RARE images were acquired in less than 5 minutes.

RESULTS AND DISCUSSION

A conventional ultra fast RARE image shown in Figure 1(a) shows an intensity gradient across the swollen polymer. However, the image contrast is not directly related to the water concentration within the system. It is thus not possible to quantify the water uptake and hence the swelling process based on this type of image. The new MRI techniques developed in this work allow us to produce the absolute water concentration, (I_0) map, and the associated T_2 and diffusion coefficient maps. The I_0 map shown in Figure 1(b) represents the total percentage of water absorbed by the polymer during the swelling process which is crucial information for the design and optimisation of controlled drug release systems. Furthermore, the T_2 and diffusion coefficient parameter maps shown in Figure 1(c) and (d), demonstrate that the traditional "gel layer" can be more precisely defined as a gel layer and a swollen glassy layer. The swollen glassy layer has been reported the literature (7) using non-quantitative optical imaging technique, but this is the first time it has been revealed using ultra-fast MRI techniques. A new modified physical model for the HPMC controlled drug release systems is formulated based on the results of this work is shown in Figure 2.

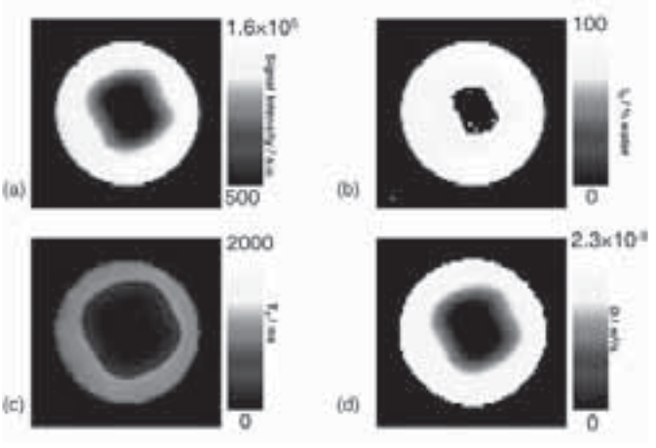

Figure 1: (a) a 64 64 RARE horizontal image taken through the HPMC/water system; the corresponding
(b) water concentration (I_0) map;
(c) T_2 map;
(d) diffusion coefficient map

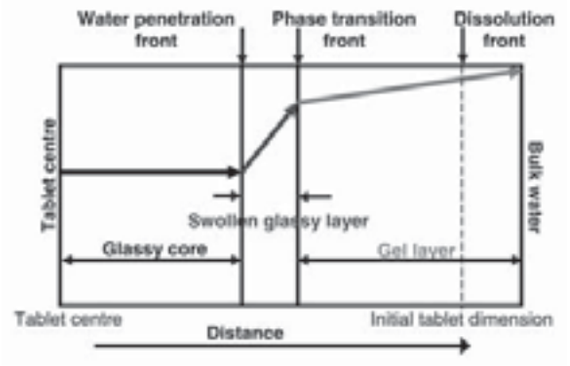

Figure 2: 1D schematic describing the phase boundaries of the swollen HPMC polymer in water

CONCLUSIONS

The results shown above suggest that the preconditioned RARE techniques are powerful tools to study the HPMC dissolution process in water solvent in a quantitative manner. We have specifically been able to quantify both water uptake and water self diffusivities within the HPMC polymer, which provides valuable information for improving the design and optimisation of such drug delivery polymers. The robustness of the preconditioned RARE technique makes it ideal for quantifying many polymer dissolution/drug release processes on ever more rapid timescales.

References

1. Rajabi-Siahboomi A. R., Bowtell R. W., Mansfield P., Davies M. C., Melia C. D.: Pharm. Res. 13, 376-380 (1996)
2. Haacke E. M., Brown R. W., Thompson M. R., Venkatesan R.: Magnetic Resonance Imaging, 1 ed., New York: John Wiley & Sons 1999.
3. Mantle M. D., Sederman A. J.: Prog. in. Nucl. Magn. Reson. Spectroscopy. 43, 3-60 (2003)
4. Richardson J. C., Bowtell R. W., Mader K., Melia C. D.: Adv. Drug Deliv. Rev. 57, 1191-1209 (2005)
5. Callaghan P. T.: Principles of Nuclear Magnetic Resonance Microscopy, 1 ed., New York: Oxford University Press 1993.
6. Hennig J., Nauerth A., Feiedburg H.: Magn. Reson. Med. 3, 823-833 (1986)
7. Gao P., Meury R. H.: J. Pharm. Sci. 85, 725-731 (1996)

OP012

Determining the Crystal Purity of Mebendazole Raw Material and Stability in Suspension by DRA-UV-Spectroscopy, ATR-FTIR Spectroscopy and ANN Spectral Modelling

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INTRODUCTION

Mebendazole, methyl 5-benzoyl benzimidazole-2-carbamate, a broad-spectrum anthelmintic drug exists in three polymorphic forms (A, B, C) with different solubilities and thermodynamic stabilities [1]. Due to its poor solubility, the more soluble, but less stable Form C is therapeutically favoured in drug formulations such as suspensions [2]. The objectives of this work were to develop simple, direct and non-destructive procedures to identify and quantify crystal purity of mebendazole raw material and to establish stability of the preferred Form C in a suspension formulation.

EXPERIMENTAL

Spectroscopy measurements were performed using diffuse reflectance techniques including Diffuse Reflectance UV (DRA-UV) and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR). Quantitative results were compared with those obtained using the X-Ray Diffraction (XRD) method. Quantitation of polymorphic forms was carried out using ANN data modeling.

RESULTS AND DISCUSSION

The DRA-UV spectra of two polymorphic forms are quite different (Figure 1) with Form C and bulk drug showing a maximum absorbance at ~320 nm and shoulder peak at ~280 nm. The spectra of Form A however show two distinct peaks at ~345 and at ~280 nm. This indicates that the principal chromophore responsible for the absorption peak at higher wavelength is changed. Moreover, Form A has a significant absorption at 355 nm, while form C and bulk drug does not absorb at this spectral region.

The ATR-FTIR absorption curves of the mebendazole raw material, polymorphic mixtures and pure Forms C and A, shows characteristic >NH- and C=0 stretching frequencies at 3404 cm^{-1} and 1718 cm^{-1} respectively (Figure 2), that can be used to distinguish between crystal form.

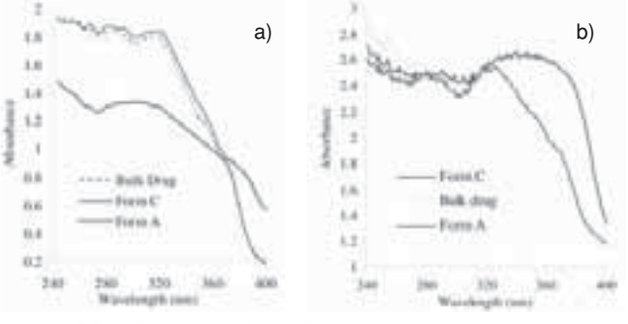

Figure 1: DRA-UV spectra of the Mebendazole bulk drug and polymorphs A and C as powders (a) and in paste (b)

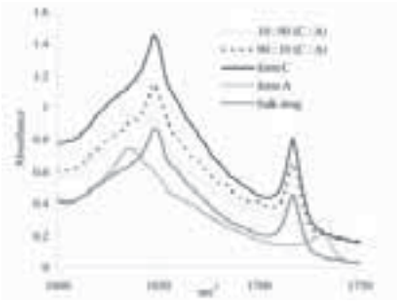

Figure 2: The ATR IR spectra of mebendazole raw material, selected crystal mixture and pure Forms C and A

Quantitation of polymorphic mixtures was carried out with ATR-IR spectroscopy and confirmed with the XRD method. Artificial neural networks (ANN) were employed as a data modelling tool. The developed ANN models confirmed that the characteristic absorptions in the IR spectral region are directly proportional to the measured amounts of mebendazole crystal forms present in the samples (r^2 >0.94 and r^2>0.97). The developed ANN models also predicted that the mebendazole raw material contained 7.21 ± 1.25% (ATR-IR data) and 10.38 ± 0.18% (XRD data) of Form A as impurity.

Stability of mebendazole in suspension was assessed using ATR-IR spectroscopy. Since mebendazole suspension contains the metastable Form C, precautions have to be taken as polymorphic conversion of Form C to more stable and less soluble form A is most likely to occur in suspension, resulting in altered bioavailability. Thus, we also wanted to asses the crystallinity of mebendazole in suspension over the period of 2 months. Results indicated that a small amount of the Form C had dissolved and recrystallised as the more stable Form A. The stability of the metastable form in suspension allowed for the observation of the simultaneous growth of both polymorphs with different growth rates. The ratio of two crystal forms however remained constant after 10 days and index of crystallinity in the suspension did not changed over investigated time.

CONCLUSION

Quantitative results obtained for binary crystal form mixtures clearly demonstrate the strong potential of ATR-FTIR technique to be used in quantitative and qualitative analysis of polymorphic content of bulk pharmaceutical materials as well as in liquid formulations.

References

1. M. Himmelreich, B.J. Rawson, T.R. Watson, Polymorphic Forms of Mebendazole, *Australian Journal of Pharmaceutical Sciences,* December (1977), **6** (4) 123-125.
2. P. J. Charoenlarp, C. Waikagul, S. Muennoo, D. Srinophakun, Kitayaporn, Efficacy of single-dose mebendazole, polymorphic forms A and C, in the treatment of hookworm and Trichuris infections, Southeast Asian J. Trop. Med. Public Health 24 (1993) 712–716.

OP013

Twin screw extrusion, compression and coating processes analyzed by TSC, micro-raman and AFM techniques

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INTRODUCTION

The temperature and pressure dependent changes of the morphology of API-s are governed by thermodynamic and/or kinetic control [1]. Kinetic control act through the changes of molecular mobility in the range around the glass transition temperature (T_g).

The morphology of drugs influences the processability and bioavailability [2], while the technology modifies or stabilizes the morphology. Solvatation, desolvatation, amorphization, crystallization or trans-crystallization may occur due to the influence of processing parameters [3]. As these influences are hardly detected and controlled in the current technologies urgent need exists, at each stages of technology for (on line/at line) analyses being able to determine the local changes of the structure of pharmaceuticals. Among other solid-phase analytical methods [4,5] vibrational spectroscopy is especially suitable for in- process monitoring and chemical imaging. Raman micro-spectrometry of 1 μm lateral resolution allows determining the local structure of components in multicomponent systems [6].

Thermally stimulated depolarization current (TSC) is a unique tool for getting an insight in the structure of amorphous materials. Changes of mobility of groups and other molecular units can be detected sensitively (thermodynamic transitions and kinetically controlled changes can be monitored this way).

In this work stabilization of the morphology of various API-s by melt extrusion has been performed varying the composition and processing parameters. Real time detection of the extrusion process allowed controlling the distribution and polymorphic transformation of API-s. The technology and composition dependent changes of Tg has been determined by TSC method. Comparison of the granulation processes in respect of the distribution of the API; non-destructive characterization of tablets and evaluation of the efficiency of newly developed humidity barrier coating belonged to the aims of the present work.

EXPERIMENTAL METHODS

Extrusion granulations were performed in Collin twin screw extruder and MiniLab HAAKE Rheomex CTW5 instrument. The latter one is

equipped with a Raman detector allowing real time control of the homogenization process. Compressing was performed by Dott. Bonapace CPR 6 computer-controlled tablet forming machine, in which the compression and rejecting forces are measured by piezo-electric sensors and data processing is done by DasyLab 8.0 softver.
The chemical structures were investigated by micro-Raman analysis using a LabRam type Jobin Yvon apparatus with frequency doubled NdYAG laser of 532 nm, power: 10 mW without intensity filter, the objective was 50x.
Thermally Stimulated Current (TSC) measurements (Setaram instrument) were performed with the following phases: *Polarization:* sample is heated up to preset temperature and is charged with 400 V/mm. *Freezing:* sample is cooled, with rate of 20°C/min, down to -150°C stabilizing the oriented dipoles. After that the electrical field is removed. *Depolarization:* heating up the sample with 7°C/min while the current, induced by the movement of the dipole segments, is measured.
Topographic images of coating layers were taken by an AFM-μTA 2990 (TA Instrument) type atomic force microscope coupled by micro- thermal analyser.

RESULTS

On line and off line methods have been elaborated for detecting structural changes at all stages of the technology of solid pharmaceuticals.
Concerning the stabilization of the morphology of API-s, by means of melt extrusion of varied composition and processing parameters, Raman analysis was used. It allowed distinguishing slight changes of amorphous structure far below the crystalline state as shown in Fig.1. Local arrangement of API in the course of various granulation processes could be compared with the aid of real time Raman detection.

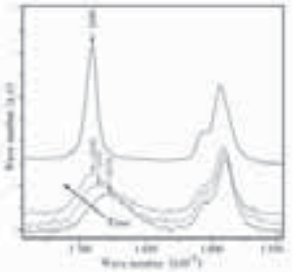

Figure 1: Shift of characteristic band of amorphous API towards crystalline reference

Stabilization efficiency of the polymer matrix is influenced by its Tg, which could be detected by TSC (see in Fig. 2) more clearly than by DSC.

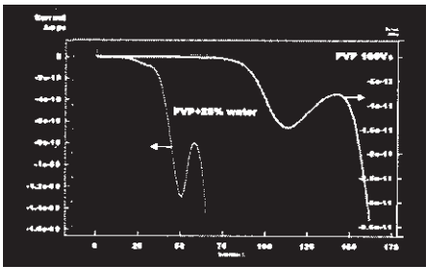

Figure 2: TSC curves of PVP and PVP+25% H2O

Non-destructive characterization of tablets has been performed by means of Raman spectrometry. Correlation has been found between the intensity of characteristic bands of Raman spectra and structure of tablets formed under controlled process of compression. The curve of correlation is shown in Fig. 3. The method allows the estimation of the strength of the tablets.

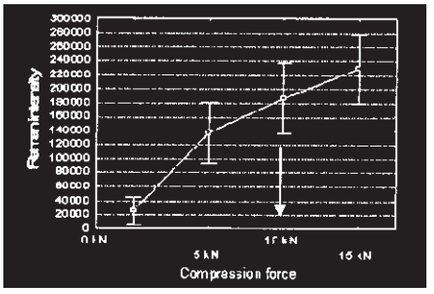

Figure 3: Relationship between the intensity of the Raman spectrum and compression force

Similar Raman method was elaborated for controlling the efficacy of the coating process. The failures of coating layers were evaluated based on AFM images. The mapping done with AFM tip allowed determining the local level of merging of the drops of the coating material.

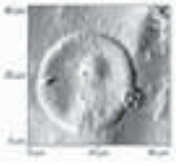

Figure 4: AFM image of the surface of coating

CONCLUSIONS

Real time Raman analysis allows detecting polymorphic changes of API during and after granulation. Tg of the matrix influences its stabilizing capability, which could be detected by means of TSC clearly. Evaluation of the micro-Raman results taken after computer-controlled tablet forming made feasible the non-destructive testing of the strength of tablets. AFM and Raman analyses promoted the understanding of the coating process.

ACKNOWLEDGEMENT

The authors acknowledge the financial supports received through the EU-6 Framework Program (NMP3-CT-2004-505637), Multihybrids (IP 026685-2) and Public Benefit Association of Sciences and Sport of the Budapest University of Technology and Economics.

References

1. A. Burger, R. Ramberger, *On the poyimorphism of the pharmaceuticals and other molecular crystals* Microchim. Acta, Wien, 1979 259-316
2. W.I. Higuchi, P.K. Lau, T. Higuchi, J.W. Shell, *Polymorphism and drug availibility. Solubility relations in the methylprednisolone system*, J. Pharm. Sci. 52 (1963) p. 150-153
3. H.G. Brittain, E.F. Friese, *Effects of pharmaceutical processing on drug polymorphs and solvates*, in: H.G. Brittain (Ed.), *Polymorphism in pharmaceutical solids*, Vol. 95, Marcel Dekker, New York, 1999, p.331-361
4. G.A. Stephenson, R.A. Forbes, S.M. Reutzel-Edens, *Characterization of the solid state: quantitative issues*, Adv. Drug Deliver. Rev. 48 (2001) p. 67-90
5. S.R.Vippagunta, H.G. Brittain, D.J.W. Grant, Crystalline Solida, Adv. Drug Deliver. Rev. 48 (2001) 43-65
6. Pelletier, M. J. :*Quantitative Analysis Using Raman Spectrometry*, Appl. Spectr. 57 (2003) 1

OP014

Preparation and in vitro characterization of anticancer drug loaded-implantable PLGA microparticles

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INTRODUCTION

Treatment of brain cancer remains a challenge despite recent improvements in surgery and drug therapy. Therefore, polar and lipid-insoluble substances such as amino neuropeptides, chemotherapeutic agents need specific carrier systems (liposomes, nanoparticles, and chimeric peptide technology etc.) to pass into the brain or implantable, biocompatible and biodegradable carrier systems (microparticles) for local delivery to the brain. Gliomas are the most common type of primary brain tumor [1,2].

The aim of this study, is to prepare an antitumour drug-loaded PLGA microparticles for treatment of malignant gliomas and characterize their particle size, yield, drug loading, surface morphology, release characteristics and cell culture studies in vitro.

EXPERIMENTAL METHODS

• Preparation of microspheres

PLGA microparticles were prepared using a modified version of an o/w single-emulsion solvent evaporation process [3]. In brief, the organic phase consisted of PLGA polymer in an acetone–dichloromethane mixture (0,5:1). The aqueous phase contained PVA solution and drug dissolved. The organic phase was emulsified with the aqueous phase by using Ultra Turrax model T25 (IKA Labortechnik, Germany) at 13,500 rpm in an ice bath for 3 min. The organic mixture was then rapidly removed by evaporation under reduced pressure at 37 °C. The particles were centrifuged at 13,500 rpm for 40 min, washed three times in distilled water and freeze-dried.

• Characterization of microspheres

• Surface morphology and particle size

The surface morphology of the PLGA microsparticles was examined by scanning electron microscopy (SEM) (Jeol-SEM 1200 EX, Japan). Microspheres were mounted on metal stubs with conductive silver paint and then sputtered with a layer of gold at 150 Å thickness using a Bio-Rad apparatus. The particle size, polydispersity of the size distribution were measured using a Malvern Zetasizer.

• Determination of drug loading

The drug loaded microparticles were separated from the aqueous suspension medium by ultracentrifugation at 13,500 rpm and 4 °C for 40 min. The amount of free drug was measured in the clear supernatant using HPLC-DAD. Reversed phase chromatography for HPLC method was conducted using a Phenomenex Bondclone C_{18} column with an isocratic mobile phase consisting of acetonitrile:water (50:50) The effluent was monitored on a DAD detector at 490 nm. Linear response ($r > 0.99$) was observed over the range of 25-200 μg ml^{-1}

• In vitro release study

The in vitro drug release studies of the doxorubicin-loaded PLGA microspheres were carried out in PBS (pH 7.4). The freeze-dried microspheres (1 mg) were dispersed in 1 mL PBS and placed in a shaker bath at 50 rpm at 37 ± 0.5°C. The in vitro release of doxorubicin was quantified by using HPLC-DAD under conditions similar to that described above.

• Cell culture

RG2 cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 0.584 g/L L-glutamine, 0.11 g/L sodium pyruvate, 50 U/ml penicillin G sodium, and 50 μg/ml streptomycin sulfate at 37 °C in a humidified incubator containing 5% CO_2. Cells were grown in 75-cm^2 culture flasks. Confluent cell monolayers were trypsinized cells were plated into 96 well, flat bottomed plates at 5x10^3 cells/100 ul/well and incubated overnight at 37 °C in an CO_2 incubator. Following day, Cells were treated with Doxorubicin solutions, Unloaded PLGA microspheres and Doxorubicin loaded PLGA microspheres in 20 μL complete medium and incubated for 48 hours in the incubator. 25 ul of MTT solution (1 mg/mL) was added to each well and waited for 4 hours. Then, medium was removed from each well and 200 μL of DMSO was added to each well. The plate was placed on a shaker for 2 minutes to dissolve the formazan in the DMSO. Following overnight incubation at 37°C, the optical densities (OD) were measured at 570 nm using a microplate reader (Spectramax Plus, Molecular Devices, UK).

RESULTS AND DISCUSSION

SEM images reveal that the microparticles are in fine spherical shape (Figure 1).

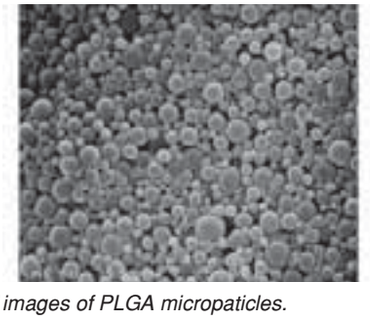

Figure 1: SEM images of PLGA micropaticles.

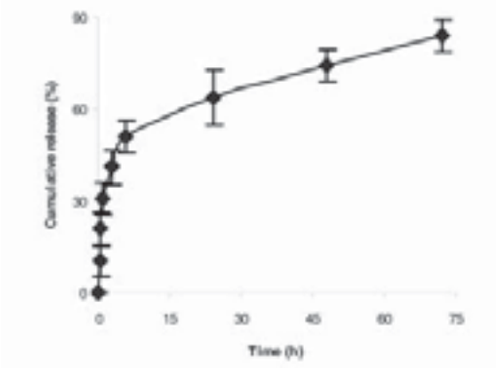

Figure 2: Release profiles of doxorubicin-loaded PLGA microparticles.

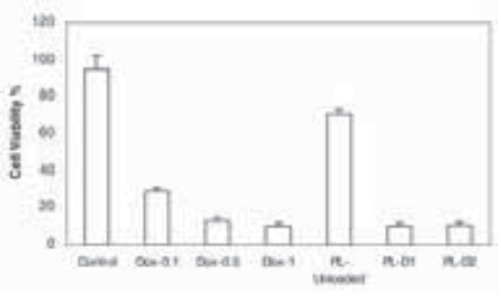

Figure 3: Viability of RG-2 according to the MTT assay after 48 hours incubation with different concentrations of several doxorubicin standards (0.1, 0.5, 1 μg/mL), doxorubicin-loaded PLGA microparticles (PL-D), and unloaded PLGA microparticles formulations (PL-unloaded).

The doxorubicin-loaded PLGA microparticles had an average diameter of 500 ± 8,5 nm. The doxorubicin encapsulation efficiency is 22,75 % which leading to final doxorubicin loading value is 0,78 %.

Fig. 2 displays the release profiles of doxorubicin from the microparticles in sink conditions. In vitro release studies showed that around 60% of the loaded drug was released into PBS by 24 h.

In the Figure 3, the MTT data shows a concentration-dependent decrease in cell line. This decrease, as seen in doxorubicin solutions, was most pronounced at low doxorubicin concentrations up to 1 M and in most cases did not change up to a doxorubicin concentration of 5 μM [2]

CONCLUSION

This study reports the preparation of doxorubicin-loaded PLGA microparticles and their characteristics. The microparticles exhibited uniform spherical shape with a mean diameter of 500 ± 6,9 nm. Doxorubicin-loaded PLGA microparticles displayed a significantly cytotoxicty towards the RG2 cells as compared to unloaded PLGA microparticles.

References

1. Drug Delivery in Cancer

1. Rainov NG, et al. Novel therapies for malignant gliomas: a local affair? Neurosurg. Focus. 15;20(4):E9 (2006).
2. De Juan BS, et al. Cytotoxicity of doxorubicin bound to poly(butyl cyanoacrylate) nanoparticles in rat glioma cell lines using different assays. J Drug Target. 2006 14(9): 614-22 (2006).
3. Songa X, et al. Pharmaceutical Nanotechnology PLGA nanoparticles simultaneously loaded with vincristine sulfate and verapamil hydrochloride: Systematic study of particle size and drug entrapment efficiency. Int. J. Pharm. 350: 320–329(2008).

OP015

Establishment and validation of an ex vivo human cervical tissue model for local delivery studies

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The human cervix is the point of origin of cervical cancer, the second most common female tumor entity worldwide. The necessary cause of cervical cancer is the infection of the cervix with high risk types of human papilloma virus (HPV). To address a local therapy with nucleic acid drugs it is important to understand the barrier properties of the target tissue to which the drug will be applied. The aim of this study was to establish and validate an *ex vivo* human cervical tissue model for delivery and permeation studies.

Permeability studies were conducted using the static Franz cell system. The cervical biopsies of an area of 0.125 cm^2 were inserted with the mucosal side oriented upwards. The transport experiments were performed at 37 °C applying a pH gradient (donor pH 6.0/acceptor pH 7.4). 40 μM [14C]Mannitol and [^{3}H]Propanolol were used. [^{14}C]Dextran 10.000 was used at a concentration of 1.5 μg/ml without the addition of unlabelled Dextran 10.000 and 40 mg/ml FITC-Dextrans were used. The diffusion studies were carried out over 24 hours. After a preincubation time of 15 minutes, the acceptor medium was sampled after 0.5, 1, 2, 4, 6, 18 and 24 h.

To determine the influence of the menopausal status of the investigated 34 patients (21 pre- and 13 postmenopausal) the Papp values were statistically evaluated for three subgroups of small molecules, hydrophilic molecules and larger molecules. These subgroups show nearly an equal distribution of patients of both menopausal status. The Papp of the various markers demonstrated that with increasing molecular weight the marker permeability decreases; an upper permeability limit between 10.000 to 20.000 Da; no significant intraindividual variability; because the

Papp values were comparable among biopsies of the same patient a significant variation of the permeability among different patient samples. A continuous difference of one log value between the Papp of Mannitol and Dextran 4.000 make them suitable as an internal marker control pair for each biopsy. The Papp values of both markers across fresh and frozen tissue are comparable.

According to these data we concluded that the human cervical tissue model has been well characterized and is suitable for delivery and permeation studies with an focus on nucleic acid drugs.

OP016

Raloxifene pre-systemic metabolic clearance in the intestinal wall and in the liver

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INTRODUCTION

Raloxifene is a selective estrogen modulator used for the prevention and treatment of osteoporosis in postmenopausal women. Recently, raloxifene has gained a lot of attention due to the discovery of its potent breast cancer preventive effects. Raloxifene exhibits a large (30-50 %) variability in pharmacokinetic parameters [1], the source of which is unknown. Its absolute oral bioavailability in humans is very low (2 %) and the absorption is relatively slow (t_{max} = 6h) [1] which is surprising for a BCS class 2 drug. Raloxifene is extensively conjugated with glucuronic acid. The intestinal wall and the liver have been recognized as the main sites of the pre-systemic metabolism. The formed raloxifene glucuronides are largely excreted into the intestinal lumen and can be cleaved back to parent raloxifene and be reabsorbed again, forming an intensive entero-hepatic and entero-enteric circulation [1,2]. Raloxifene is excreted out of the body almost exclusively through the intestine with feces [1]. The main UGT isoforms responsible for glucuronidation of raloxifene are thought to be UGT1A1, 1A8 and 1A10. They catalyze the formation of two metabolites: raloxifene-6-glucuronide (M1) and raloxifene-4'-glucuronide (M2). Among different authors, a uniform conclusion has not been reached regarding the relative contributions of the intestine and the liver in raloxifene metabolic clearance.

The primary objective of our study was to investigate the pre-systemic metabolism and transport of raloxifene and to identify the key processes that are most likely to affect its pharmacokinetics.

EXPERIMENTAL

The models were chosen to cover the absorptive and metabolic processes; both of which are vital to raloxifene pharmacokinetics.

a) The first and the least complex model used were the incubations with human recombinant UGTs (1A1, 1A8 and 1A10), expressed in Supersomes™.

b) Porcine liver and intestinal microsomes were prepared from fresh deep-frozen tissues. Human liver and small-intestinal microsomes were purchased from In Vitro Technologies (Chicago, IL). Similarly to the Supersome™ experiments, the microsomes were incubated with increasing raloxifene concentrations. The intrinsic metabolic clearances were calculated from the formed metabolite concentrations by the use of a substrate inhibition model:

$$\frac{v_0}{[S]} = \frac{V_{max}}{K_m + [S] + \frac{[S]^2}{K_{SI}}} \qquad Cl_{int} = \frac{V_{max}}{K_m}$$

c) The most complex experimental model used was the Sweetana-Grass type diffusion chamber, where a segment of a viable porcine intestinal tissue separates the donor and acceptor compartments. The experiments were performed in the absorptive (mucosal to serosal, M-S) and secretory (serosal to mucosal, S-M) direction.

$$P_{APP} = \frac{J}{C_0} \qquad Cl^{M,S}_{INT,M1,M2} = \frac{\theta^{M,S}_{M1,M2}}{C_0}$$

All samples were assayed for M1, M2 and Ral concentrations by LC-MS-MS [3].

RESULTS AND DISCUSSION

a) and b) Supersomes™ and microsomes

A very high raloxifene glucuronidation rate observed in the liver microsomes demonstrates the importance of this organ in raloxifene clearance *in vivo*, especially considering the high liver blood flow and the high enzyme content. Further important results from the subcellular models are the pronounced substrate inhibition effects (Figure 1) and the evident similarity in raloxifene glucuronidation kinetics between the human and the pig species reflected by the very close K_M, K_{SI} and V_{MAX} values determined in both species (Table 1).

These results warranted the use of a porcine duodenal tissue for the transport and metabolism experiments instead of the human tissues.

c) A significantly greater duodenal permeabilities were observed in the absorptive direction (M-S) than in excretory direction (S-M) of raloxifene: (5,7 ± 0,6) *10^{-6} compared to (0,92 ± 0,3) *10^{-6} cm/s, respectively, p=0,0005 (Figure 2). The pronounced differences between the

M-S and S-M raloxifene permeabilities were attributed to an active absorptive transport.

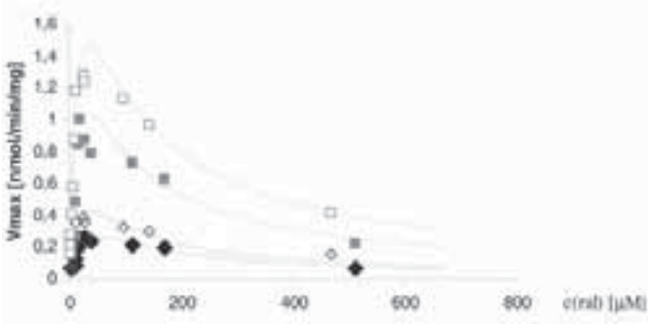

Figure 1: Fitted (solid lines) glucuronidation kinetics of raloxifene to M2 (upper two curves) and to M1 (lower two curves) in human (empty squares) and in pig (full sqares) intestinal microsomes.

Table 1: Results of the raloxifene glucuronidation experiments with human and porcine liver and intestinal microsomes

	liver microsomes			
	M1		M2	
origin	human	porcine	human	porcine
K_M [µM]	376 ± 224	1071 ± 48	142 ± 55	114 ± 1,8
V_{max} [nmol/min/mg]	6,16 ± 3,09	11,92 ± 1,1	3,56 ± 0,99	6,97 ± 0,12
K_{si} [µM]	180 ± 121	88 ± 12	226 ± 99	679 ± 78
Cl_{int} [µL/min/mg]	16,3 ± 17,9	11,1 ± 1,5	25,1 ± 16,9	61,1 ± 3,7

	intestinal microsomes			
	M1		M2	
origin	human	porcine	human	porcine
K_M [µM]	170 ± 29	389 ± 47	378 ± 51	84 ± 8,5
V_{max} [nmol/min/mg]	1,41 ± 0,022	2,41 ± 0,09	8,11 ± 1,21	2,75 ± 0,25
K_{si} [µM]	104 ± 9	24 ± 6	57 ± 11	90 ± 14
Cl_{int} [µL/min/mg]	8,3 ± 1,6	6,2 ± 1,0	21,5 ± 6,1	32,7 ± 6,3

In all metabolism and transport studies across intestinal mucosa, an extensive metabolite formation and excretion was observed. The highly polarized metabolite excretion was also attributed to an active transport process to apical (MRP-2) and to basolateral side (MRP-1, MRP-3 or OAT). The exact transporters involved in raloxifene uptake and glucuronide excretion are yet to be confirmed in our laboratory. Furthermore, the glucuronide M2 excretion rate was significantly greater in the S-M experiments (p=0,02, Figure 2, higher grey bars).

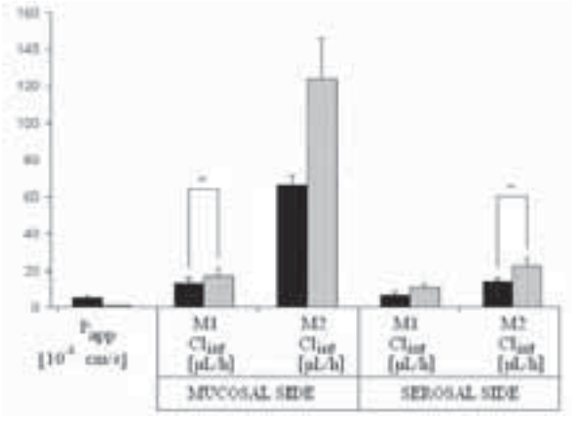

Figure 2: Apparent permeability of raloxifene and the formation clearances of M1 and M2 on mucosal and serosal side. Black and grey bars represent M-S and S-M experiments, respectively (N=4).

This observation is consistent with a lower intracellular (IC) raloxifene concentration in the S-M experiment because of an active raloxifene M-S transport, depleting the IC compartment of raloxifene. The lower raloxifene concentration in turn leads to a higher conjugation rate because of the lesser substrate inhibition (Figure 1).

CONCLUSION

Our results have shown a strong raloxifene conjugation and glucuronide excretion activity in duodenal mucosa, representing a tough raloxifene absorption barrier. Additionally, the liver was also recognized as a very important organ for raloxifene conjugation. Therefore, our results explain the low raloxifene bioavailability (2 %) and the relatively slow *in vivo* absorption rate of a highly permeable drug. Furthermore, we have identified and explained a transporter-conjugative enzyme interplay with an integrated use of complex and simple *in vitro* models. Moreover, the methodology presented here could well be applied to other substances with a high pre-systemic clearance as well.

References

1. Hochner-Celnikier D. Pharmacokinetics of raloxifene Eur. J. Obstet. Gynecol. Reprod Biol; 85(1):23-9 (1999).
2. Jeong EJ. et al. Species- and disposition model-dependent metabolism of raloxifene in gut and liver: role of UGT1A10. Drug Metab Dispos; 33(6):785-94 (2005).
3. Trontelj J. et al. Development and validation of a liquid chromatography–tandem mass spectrometry assay for determination of raloxifene. J Chrom B, 855; 220–227B, (2007).

OP017

In-situ forming lyptropic liquid crystals as drug delivery systems

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INTRODUCTION

During the past decade, there has been great interest in lyotropic liquid crystalline systems (LLC) as delivery systems in the cosmetic and chemical industries and also in the field of pharmacy The reasons for this interest include the extensive similarity of these colloid systems to those in living organisms. LLC systems are characterized by the properties of both liquids and solids, i.e. they exhibit in part a structure typical of fluids and also the structured, crystalline state of solids. Liquid crystal states with various structures, capable of being transformed into each other in a definite sequence under certain circumstances, are also called mesophases . They are usually formed from water and one or two surfactants and possibly cosurfactants, in the definite proportions of the given components, with low energy input or by means of spontaneous structural organization; their production is therefore relatively simple and energy-saving.They are thermodynamically stable.

In situ forming LLC systems undergoing transitions from a low to a high viscous state gained significant interest among formulators within the pharmaceutical field as drug delivery vehicles for dermal, nasal, ocular, oral, buccal, vaginal, rectal and parenteral administration. Factors inducing gelation include alterations in the pH, ionic strength, solvent concentration and temperature. In situ gelling systems possess several advantageous properties. In certain cases the systematically applied active agent can not achieve the required concentration and/or retention time at the site of action. This drawback can be markedly reduced by incorporating the active ingredient into topically applied in situ gelling drug delivery systems. Due to their low initial viscosity these preparations can be easily injected into site of application where they undergo a sol-gel transformation by meeting physiological conditions. The increased viscosity ensures the desired retention time while the local release of drug the adequate concentration. In this way site-specific action, prolonged delivery periods, decreased drug dosage with concurrent reduction in possible undesirable side effects common to most forms of systemic delivery, and improved patient compliance and comfort can be achieved.

The aim of our research was to develop LLC preconcentrates consisting of oil and surfactant, which contain components with good physiological tolerance and spontaneously form lyotropic liquid crystalline phase of high viscosity in an aqueous medium. This mixture can be injected into the body pocket, where it is transformed into a liquid crystalline phase through water absorption, and the preparation is prevented from flowing out of the pocket by its great viscosity, while drug release is controlled by the liquid crystalline structure.

MATERIALS AND METHODS

Materials

Metronidazole-benzoate (Ph. Eur. 4) was chosen as the active agent. 3.5 % of the drug was dissolved in the samples. The carrier was the 4:1 mixture of a non-ionic surfactant: Cremophor EL (Polyoxyl 35 Castor Oil USP/NF) or Cremophor RH40 (Polyoxyl 40 Hydrogenated Castor Oil USP/NF) and Isopropylmyristate (Ph. Eur. 4). Both non-ionic surfactants are tolerated well by tissues, have low toxicity values, and their HLB value is 14-16.

Sample Preparation

The oil-surfactant mixture was homogenised with a magnetic stirrer at room temperature. The 3.5 % of metronidazole-benzoate was dissolved in this mixture with a Heidolph Diax (HEIDOLPH Instruments GmbH & Co.) mixer.

Investigations

The structure of the samples was examined with a polarization microscope (LEICA Q500 MC) at room temperature.

Rheological Investigations

Rheological measurements were carried out with a RheoStress 1 HAAKE instrument. A cone-plate measuring device was used in which the cone angle was 1 degree, and the thickness of the sample was 0.048 mm in the middle of the sensor. The measurements were performed at room temperature. The samples were kept in a space saturated with water vapour during measurement in order to prevent evaporation. The linear viscoelastic range was determined in the first step by examining the complex modulus as the function of shear stress at a given frequency (1 Hz). Based on these experiments, the value of shear stress was set at 2.5 Pa during the dynamic test as this value was always within the linear viscoelastic range, then the values of the storage and loss moduli were examined as the function of frequency.

Water up-take investigation

The water absorption mechanism of the samples was examined with the instrument used for determining the Enslin number. 1 g of waterfree sample was placed on the G1 glass filter of the instrument filled with bubble-free water, and then the quantity of the absorbed water was measured as the function of time.

Drug Release Studies

The in vitro drug release studies were performed with a vertical diffusion cell (Hanson Microette System, Hanson Research Corporation). 0.4 g of waterfree sample was placed as a donor phase on the Porafil membrane filter, the pore diameter of which was 0.45 micrometer. Alcohol of

10 w/w% was used as an acceptor phase because of the poor water solubility of metronidazole-benzoate. The measurement was performed at 37 °C for 6 hours. The quantitative measurement of metronidazole-benzoate was carried out with a UV spectrophotometer.

RESULTS AND DISCUSSION

The knowledge of the mechanism of water-uptake process may be important, because the direction of the flow caused by water absorption is opposed to drug liberation in this way it can influence the degree of drug release. The structure of the forming gel during the water uptake can influence the drug release too. The water absorption of water-free compositions was investigated using different surfactants and oils (Fig.1).

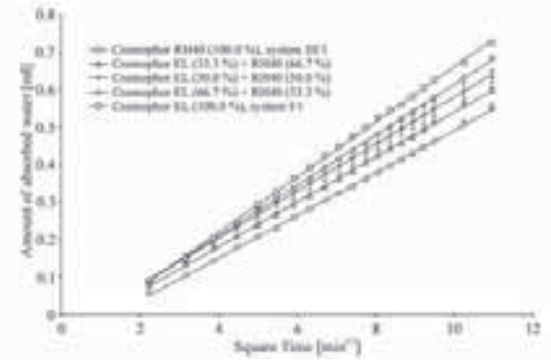

Figure 1: Water absorption as the function of square root time.

To be able to study the effect of water content exerted on rheological behaviour more precisely, the G' and G" values were investigated. Based on the Fig.2, obtained as the fraction of G" over G'(loss tangent), can be seen the effect of different water content on the elastic and plastic behaviour of the samples.

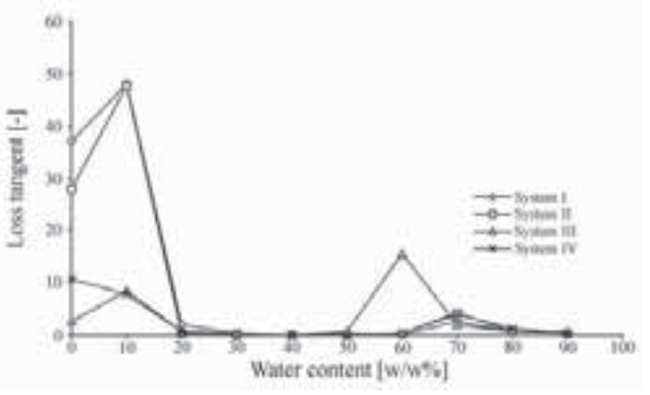

Figure 2: Values of loss tangent in the function of water content

In conclusion, the investigated water-free systems characterized by low viscosity values and pharmaceutically acceptable components are promising vehicles for the formulation of injectable in situ gelling drug delivery systems.

OP018

Nanosuspension formulation to improve the dissolution rate of Meloxicam

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INTRODUCTION

Solubility problems pose a major challenge for the pharmaceutical industry as concerns the development of new pharmaceutical products. Studies of poorly soluble drugs have demonstrated that a particle size reduction can lead to an increase in dissolution rate and higher bioavailability. During the last ten years, nanoparticle engineering processes have been developed and reported for pharmaceutical applications. In addition to overcoming issues of solubility, nanosuspensions (NSs) allow a higher mass per volume drug loading in comparison with drug solutions [1, 2].
The aims of our study were to investigate the feasibility of nanosuspension preparation, to study the effects of different preparation methods and added stabilizers on the formulated nanosuspensions. Furthermore, the prepared (NSs) were transformed into dry powdered products, either by freeze-drying or by spray-drying, and investigated the rate of dissolution of dried samples. Our model drug was Meloxicam (MEL) a nonsteroidal anti-inflammatory drug with strong analgetic and anti-inflammatory effects.

According to the biopharmaceutical classification system, MEL is a Class II drug, since it is poorly water-soluble and well permeable. Therefore, increase in dissolution rate will result in increased biological availability.

MATERIALS AND METHODS

• Materials

The drug meloxicam was from EGIS Ltd., (Budapest, Hungary). Lutrol F68 (poloxamer 188) and polyvinylpyrrolidone (PVP) K25 were from BASF (Germany), Tween 80 (polysorbate 80), benzyl alcohol from Fluka (Switzerland), and ethyl acetate from Merck (Germany). Water was purified by reverse osmosis.

• Sample preparation

MEL nanosuspensions were prepared from emulsions containing partially water-miscible organic solvent: ethyl acetate or benzyl alcohol.

The drug (20 mg) was dissolved in 20 ml of ethyl acetate, poured under stirring at 8000 rpm with Ultra Turrax T 25 (Janke & Kunkel, IKA Labortechnik, Germany) into 140 ml 0.5% aqueous solution of Tween 80 followed by high pressure homogenization (APV-2000, Invensys, Denmark) at pressure of 800 bar for 5 min, diluted with 160 ml of water and further homogenized for 5 min.

MEL (20 mg) dissolved in 9 ml of benzyl alcohol was poured into 64 ml 0.5% poloxamer 188 aqueous solution, and sonicated for 3 min (amplitude 30%, 500 W Model, Cole-Palmer Instrument Co., UK Ltd.). Emulsion was diluted with 200 ml of water and further sonicated for 3 min.

Reference samples (REF) of same composition were prepared using magnetic stirrer (Table I).

NSs were transformed into dry product either by spray-drying (SPD)(Mini Spray Dryer B-290, Büchi, Switzerland) or by liophylization (LIO)(Crist Beta 1-8 K, Germany). Trehalose (6 g per sample) was used as a redispersant or lyoprotectant.

Table 1: The process-parameters of sample praparation

Sample	Organic solvent	Stabilizer	Homogenization procedure	Drying method
SPD-NS	ethyl acetate	Tween 80	HPH	spray-drying
SPD-REF	-	Tween 80	magnetic stirrer	spray-drying
LIO-NS	benzyl alcohol	Poloxamer 188	HIUS	lyophilization
LIO-REF	-	Poloxamer 188	magnetic stirrer	lyophilization

• Sample characterization

Particle size and size distribution of nanosuspensions and reference samples was determined using Zetasizer 3000 (Malvern Instruments, Worcestershire, UK) and Mastersizer (Malvern Instruments, Worcestershire, UK). The particle *morphology* was determined by scanning electron microscopy (SEM; SUPRA 35 VP, Carl Zeiss). *Drug content* and *in vitro dissolution* rate of MEL were determined using the dissolution apparatus (Erweka DT 6, Germany). The rotation speed of the paddles was 100 rpm. 900 ml of phosphate buffer solution with pH of 7.4 ± 0.1 (Ph. Eur. 6) at 37 ± 0.5 °C was used as a dissolution medium.

RESULTS AND DISCUSSION

• Influence of organic solvents and stabilizers

The choice of organic solvent for NS preparation is crucial factor influencing final particle size and was based on preliminary experiments. Ethyl acetate has higher water miscibility compared to benzyl alcohol, however benzyl alcohol is better solvent for MEL. The results of tested different stabilizers in various concentrations showed that. Tween 80 and Poloxamer 188 in increasing concentrations yielded smaller particle size and polydispersity index, whereas PVP K-25 did not exert such an effect. The smallest particle size (~290 nm) in nanosuspensions was achieved using 0.5% Tween 80 or poloxamer 188 as stabilizer and benzyl alcohol as an organic solvent. Therefore this composition was chosen for further research work. Table II presents mean particle size of MEL samples, showing that the raw drug and reference samples have average particle size (d_{50}) in micrometer range, whereas particle size of both NSs is approximately houndred times smaller.

Table 2: Particle size distribution after dispersion of pure MEL or MEL in different dried samples in water

Samples	d (μm)		
	10%	50%	90%
MEL	*24.80*	*85.39*	*237.92*
SPD-NS	0.140	0.460	2.71
SPD-REF	5.62	42	50
LIO-NS	0.168	0.530	3.6
LIO-REF	22.83	59.17	68.87

From these results we can conclude that using Ultra Turrax alone is not enough to prepare NS from drug dispersion.The SEM pictures revealed a significantly morphologycal difference between the dried products.

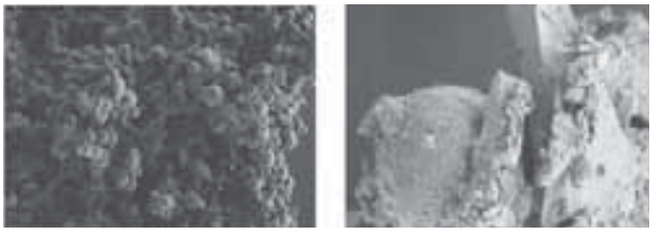

Figure 1: SEM pictures of SPD-NS and LIO-NS

• *In vitro* dissolution

Dissolution of NS formulations showed significantly higher dissolution rate compared to references, since practically all drug dissolved in first 5 min (Fig. 2).

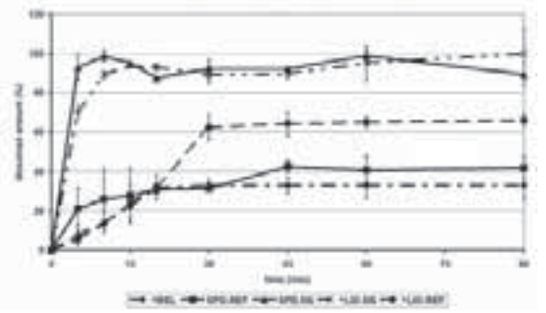

Figure 2: Dissolution curves of MEL and products

CONCLUSIONS

In conclusion, there is a statistical significant difference between the dissolution rate for pure MEL in 85 m size and for MEL in nanosized particles. When solvents for preparation of nanosuspensions are compared the size of produced nanoparticles is similar. The improved dissolution rate of MEL could improve its analgetic effect in the therapy.

Acknowledgements: This work was supported by the Hungarian-Slovenian Bilateral Intergovermental Cooperation Programme (HU-SLO-7/2006).

References

1. Ambrus, R., Pomázi, A., Aigner, Z., Kocbek, P., Kristl, J., Szabó-Révész, P. *Gyógyszerészet* (2008) Vol. 52, 259-264
2. Kocbek, P., Baumgartner, S., Kristl, J. *Int. J. Pharm.* (2006) Vol. 312, 179-186

OP019

Characterization of nanocrystal formulations which contain poorly water soluble drug, ezetimibe

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INTRODUCTION

Many new drugs can be classified an Class II drugs according to the Biopharmaceutical Classification Systems (BCS). Drug, which are Class II, are poorly/not soluble in water however their permeability is high, so when they are dissolved, they are easily absorbed. The model drug ezetimibe is a white crystalline powder and Class II drug. It is the first marketed lipid lowering drug that inhibits intestinal uptake of dietary and biliary cholesterol without affecting the absorption of bile acids, triglycerids, fat-soluble vitamins and is used in the treatment and prophylaxis of atherosclerosis. It belongs a family of azetidinone compounds. Its chemical structure is (3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone. Poor solubility of the drug is associated with poor dissolution rate and thus low oral bioavailability. [1,2,3,4].

The purpose of this study is to increase the spesific surface area of the drug following particle size reduction and to enhance ezetimibe solubility and dissolution charecteristics by preparing drug nanocrystals. Moreover, characterization of nanocrystal formulations, prepared by several techniques, such as; homogenization, co-precipitation and heat drying, were carried out.

EXPERIMENTAL METHODS

• Materials

Poloxamer F127 was obtained from Uniqema (Belgium) and Ezetimibe was a kind gift from Dr. Reddy's Laboratories (India).

• Preparation of Formulations

Co-precipitation (BC): A solution of ezetimibe and poloxamer F127 was prepared in ethanol. The solvent was then removed under reduced pressure at 40 °C and the precipitate was desiccated until use.

Heat-drying (EK): A solution of ezetimibe and poloxamer F127 was prepared in acetone. The solvent was evaporated at room temperature and the mixture was dried in a 50 °C oven.

Ultraturrax mixing (UT): A suspension of ezetimibe and poloxamer F127 was prepared in distilled water and mixed with an Ultraturrax® T25 Basic (IKA Labortechnik, Germany). The dispersion medium was then removed by lyophilization.

Ultrasonic Probe (UP): A suspension of ezetimibe and poloxamer F127 was prepared in distilled water and mixed with an Ultrasonic Probe. The dispersion medium was then removed by lyophilization.

Hand Homogenization (EH): A suspension of ezetimibe and poloxamer F127 was prepared in distilled water. The formulation was then homogenized with a Hand Homogenizator. The dispersion medium was then removed by lyophilization.

Physical Mixture (FK): Ezetimibe and poloxamer F 127 was mixed using a mortar.

• Dissolution Studies

In vitro dissolution studies were performed using SOTAX dissoltion apparatus. The dissolution studies were carried out utilizing the USP apparatus II (pedal) method. A 10 mg of the formulations corresponding to 5 mg of the drug was placed in the cells. Three dissolution media were used in this work. First dissolution media contained 900 ml of 0.15% SDS / 10 mM pH 7.0 phosphate buffer (DM1), second dissolution medium contained 500 ml 0.15% SDS in 50 mM pH 4.5 acetate buffer (DM2), and third one contained 500 ml 0.5% SDS in 50 mM pH 4.5 acetate buffer (DM3) all stirred at 37 ± 0.5 °C with 50 rpm rate. A 10 mg of ezetimibe powder was also tested as a control. A 5 ml sample was withdrawn at each of the predetermined time intervals, and the amount of the drug was determined by HPLC.

• HPLC Analysis

A validated HPLC method was utilized for the quantification of ezetimibe [5]. The mobile phase comprised water (0.05% w/v 1-heptane sulfonic acid, pH 6.8) and acetonitrile (30:70 v/v). A SphereClone ODS column (80 ºA, 5 m, 150x 4.6 mm) was used and the flow rate was set to 0.5 ml/min with an injection volume of 20 l during the experiment. The analysis was carried out at 232 nm and the retention time of the drug was determined to be 4.4 min

• Characterization of Formulations

1. DSC Analysis

Differential Scanning Calorimeter (DSC) was used to determine the degree of crystallinity and melting of ezetimibe, Pluronic F 127 and the formulations. Thermal properties of the powder samples were invastigated using a DSC Q 100 (TA Instruments, USA). Approximately, a 10 mg sample in a aluminum pan. A heating rate of 10 °C/ min was employed in the range 10-250 °C. Analyses were performed under a nitrogen purge at 1.5 bar.

2. X-ray Analysis

X-ray diffractograms of each formulation, Pluronic F 127 and ezetimibe were recorded using a X-Ray Diffractometer (Ultima, Japan). Standart runs using a 40 kV voltage, a 40 mA current and a scanning rate of 0.02° min^{-1} over a 2 ~ range of 0 - 40 ° were used.

3. FT-IR Analysis
Samples were analysed using a Fourier Transform Infrared Spectrometer (Perkin Emler, USA). The infrared spectra were detected over the wavenumber range of 4000 - 650 cm^{-1}.

RESULTS AND DISCUSSION

• Dissolution Studies

The dissolution profiles of ezetimibe in different formulations, powder and physical mixture form are given in Table 1[6].

Table 1: Amount of drug dissolved (%) at the end of 45 min from the formulations in different dissolution media (DM: dissolution media; EZE: ezetimibe powder; FK: physical mixture).

	Amount of drug dissolved (%)						
DM	EH	UP	UT	EK	EZE	FK	BC
DM1	91	77	100	41	25	20	32
DM2	55	63	57	25	23	24	23
DM3	87	74	65	60	53	49	44

• Formulation Characterization

1. DSC Analysis

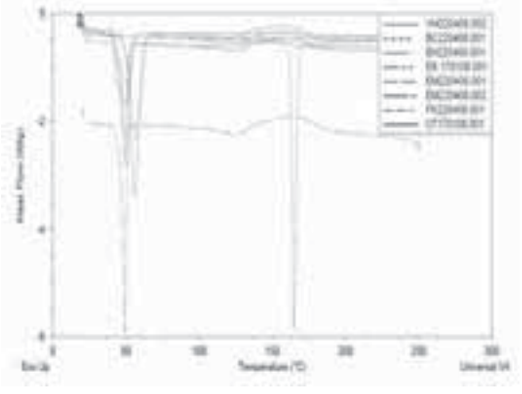

Figure 1: DSC termograms of ezetimibe (EM), Pluronic F127 (YM), physical mixture (FK) and nanocrystal formulations (BC, EK, UT,UP, EH)

2. X-ray Analysis

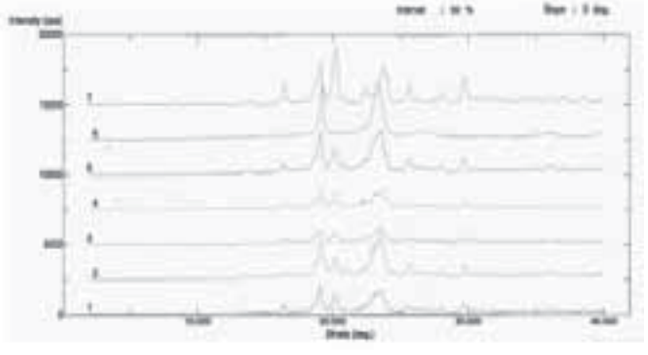

Figure 2: X-ray patterns of ezetimibe (7), Pluronic F127 (6) and nanocrystal formulations; BC (5), EK (2), UT (1), UP (4), EH (3).

3. FT-IR Analysis
FT-IR analyses were performed and similar spectra were found for ezetimibe and nanocrystal formulations.

CONCLUSION

X-ray diffraction patterns showed that drug was covered with Pluronic F 127 and this prevented the aggregation and provided a faster dissolution rate. Thus, intensity of the X-ray peaks and dissolution profiles appear to be inversely related. DSC analyses results indicate that ezetimibe and Pluronic F 127 interacted with each other in all formulations. FT-IR spectra showed that structure of formulations were the same regardless of the preparation method. These preliminary results obtained indicate that the dissolution profile of ezetimibe, a poorly water-soluble drug, could be improved by several methods, such as; ultrasonic probe and hand homogenization. The results could be attributed to the increased solubility of the drug due to the presence of the surface active agent, poloxamer F127 and the method of preparation.

ACKNOWLEDGEMENT

This project is supported by a grant from the TUBITAK Scientific and Technologic Research Project Support Program (SBAG-3710, No:107S216).

References

1. Teddy Kosolou, *et. al.* Clin Pharmacokinet, 44 (5); 467-494 (2005)
2. Novel Crystalline Form of Ezetimibe and Processes for the preparation thereof, US 2006 / 0234996 A1 (2006)
3. Harry R. Davis, International Congress Series, 1262; 243-246.
4. H. De Waard, *et. al.*Journal of Controlled Release, Article in press (2008)
5. R. Sistla, *et. al.* Journal of Pharmaceutical and Biomedical Analysis, 39 (3-4); 517-522 (2005)
6. T. Gülsün, *et. al.* Application of Nanotechnology for the Improvement of Dissolution Properties of a Poorly Water-Soluble Drug, Ezetimibe, 6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology (2008)

OP020

Development of release testing procedure for diclofenac sodium slow-release tablets

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INTRODUCTION

Dissolution/release testing is very important step in the prediction of *in vivo* drug delivery system and drug itself behaviour, i.e. in the prediction of drug plasma profiles after its administration in a suitable delivery system. Thus, the conditions in which release testing is performed are very important. The most frequently used systems for dissolution testing are official apparatus with basket or paddle stirring elements. These apparatus are an excellent tool for quality control of drug delivery systems. However, some of the conditions used are not close to physiological conditions what might represent a problem in predicting *in vivo* behaviour of drug delivery systems which are sensitive to these specific conditions.

In the scope of the present work diclofenac release from commercially available slow - release tablets was evaluated. Apparatus and conditions of testing were chosen on the basis of their ability to simulate *in vivo* situation with the purpose to obtain release profile close to *in vivo* absorption profile found in literature.

EXPERIMENTAL METHODS

Commercially available slow-release tablets containing 100mg of diclofenac sodium were evaluated.

Release experiments were performed using flow-through apparatus with conditions close to those *in vivo* (developed at the Faculty of Pharmacy, Ljubljana) and apparatus 2 acc. to USP, i.e. apparatus with paddle (VanKel 7000, VanKel Technology Group, Cary, NC, USA).

• Flow-through method

Volume of medium in 150mL beaker was 40mL, temperature 37°C and flow rate 2mL/min. The stirring rate, performed by a magnetic stirrer, was 50 rpm. Additionally, some modifications related to the movement of the dosage form were used in all experiments. Constant flow was maintained throughout the whole period of experiment. Medium was pumped out of beaker through 71 m mesh into defined volume of 0.1M NaOH to enable dissolution of undissolved diclofenac particles. The concentration of a drug was determined spectrophotometrically by measuring absorbances at 276 nm. Media were chosen according to fasted (HCl pH 1.2 and phosphate buffer pH 6.8) or fed state (citrate buffer pH 4.0 and HCl of different concentrations).

• USP, Apparatus 2

Volume of medium was 1000mL, temperature 37°C and rotational speed of the paddle 100 rpm. Tablet was firstly exposed to artificial gastric juice (pH 1.2) and then the whole medium was exchanged with artificial intestinal juice (pH 6.8). The amount of drug released was determined spectrophotometrically in the samples, which were automatically collected during the experiment.

RESULTS AND DISCUSSION

Two systems were used for release testing, official USP apparatus with paddle (app. 2) and the flow through system, developed in our laboratories. These two systems differ in mechanical forces which influence the dosage form during the testing and in other experimental conditions; with conditions in newly developed flow-through system we wanted to be as close as possible to physiological conditions. In literature (1) plasma profile after administration of slow-release diclofenac tablets was found and absorption profile of unchanged drug was calculated. In the study the tablets were administered after overnight fast before breakfast.

The first two series of experiments were performed on both systems considering residence time in artificial gastric medium 1 hour. Release results in comparison with absorption profile are shown in Figure 1. Lag-times are too long in both cases and on USP system too low amount of drug was released in six hours. In the scope of further work different conditions, like ionic strength and surfactants, were additionally varied in physiological intervals, however, none of these variations changed the release profiles to such a degree to be close to absorption profiles. To shorten lag-times, gastric medium residence time was shortened on both systems, but again without a significant improvement of release profiles. Shorter times in gastric medium were also chosen on physiological basis. An additional literature search on fasted gastric residence times of tablets namely showed that a certain percentage of tablets (in population) empty the stomach in relatively short time (2).

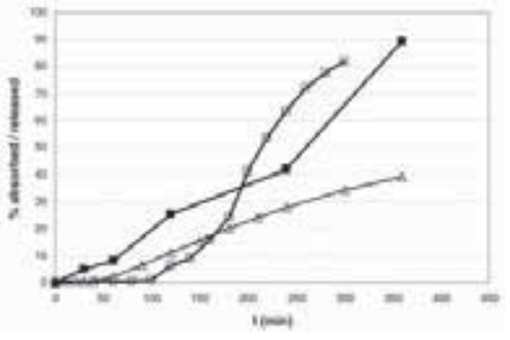

Figure 1: Absorption profile (filled squares) of unchanged diclofenac and release profiles on USP 2 apparatus (triangles) and on flow-through system (open squares) under usual fasted conditions.

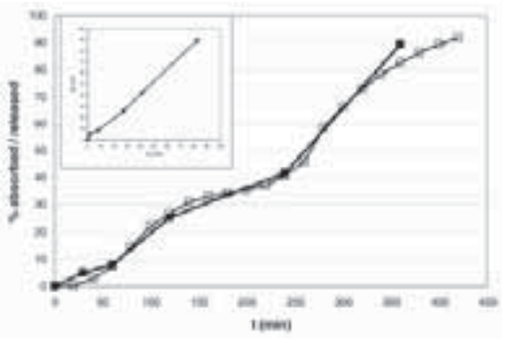

Figure 2: Release profile on flow-through system at simulated fed gastric pH values (open squares) in comparison with absorption profile (filled squares) of unchanged diclofenac. Inserted figure shows in vitro-in vivo correlation.

Further research work was focused on gastric pH values in fasted/fed state and on their influence on the dissolution/ release of diclofenac. The fact is that pH values in stomach vary significantly with the presence of food. In *in vivo* study (1) tablets were taken in fasted state, but the breakfast was ingested afterwards. So the tablets were probably exposed to pH values in stomach which were changed with regard to the presence of food. Another fact is that diclofenac has very low solubility in acidic medium due to formation of cyclic form (3). The pH value of media is very important because diclofenac's solubility increases at higher pH values. In *in vivo* study tablets were taken before breakfast, but unfortunately no information about caloric value of the meal is given. pH in fed stomach is dependent on caloric value of ingested food and we decided to search the literature for pH values in stomach after meal of medium caloric values (4). However, these values vary in different parts of stomach and we do not have data about resting times of tablets in a specific part of the stomach. In spite all these facts, approximate pH profile was constructed that might simulate pH values to which tablet is exposed after taking an "average" breakfast. Tablets were kept at pH 4.0 for 10 minutes and then pH was slowly decreased to the value below 2.0. This value was maintained for a certain period of time. 3 hours after the beginning of experiment the inflow medium was changed with buffer pH 6.8 thus simulating the emptying of the tablet from the stomach into the duodenum and small intestine. Due to frequent changing of media the experiments were performed only on flow - through system and the result is shown on Figure 2.

CONCLUSION

Good *in vitro – in vivo* correlation for diclofenac sodium slow – release tablets was obtained in flow – through system under some of important experimental conditions close to those *in vivo* in fed state. Especially great influence is ascribed to simulated pH values in stomach after ingestion of food and to mechanical influences which are different in flow – through system in comparison with USP paddle apparatus.

References

1. Arcelloni C et al., J Chromatogr B, 2001,736,195-200
2. Locatelli et al., Submitted to CESPT, Ljubljana, 2008
3. Palomo ME et al., J Pharm Biomed Anal 1999,21,83-94
4. Savarino V. et al., Dig Dis Sci 1996,41,1379-1383

OP021

Optimization of drug release from compressed multiparticulate units using generalized regression neural network

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INTRODUCTION

In this study development of diclofenac sodium extended release matrix pellets compressed into tablets was optimized using Generalized Regression Neural Network (GRNN). The ratio of matrix substance, Carbopol 71 G in prepared multiparticulate units and crushing strength of the tablet (i.e. compressed pellets) were screened as the most important –causal factors- responsible for release of diclofenac sodium in 8 hours. The purpose of the applied GRNN was to model the effects of these two causal factors on the in vitro release profile of the diclofenac sodium from compressed matrix pellets. The aim of the study was to optimize drug release in manner wich enables following release during 8 hours: 1h: 15-40%, 2h: 25-60%, 4h: 35-75%, 8h: >70% of diclofenac sodium to be relased.

EXPERIMENTAL METHODS

Materials

Diclofenac sodium (Novartis, Switzerland), Carbopol® 71 G (Gattefosse, Switzerland), Avicel® PH 101 (FMC, USA), Magnesium stearate (Sandoz, Germany) and Aerosil® (Degussa, Germany).

Methods

• *Pellets preparation*

Pellets were prepared by direct pelletisation in a rotary fluidized bed granulator GPCG1 (Glatt, Binzen, Germany) with different Avicel® PH 101: Carbopol® 71 G ratio (varied from 5.86% to 34.14% of Carbopol®

71 G), using water as granulated liquid. The process of pelletisation was controlled by a computer program.

• *Pellets characterization*

The pellets F_1-F_{10} were characterized on particle size distribution by laser diffraction using *Malvern, Mastersizer*, density measurement was performed: true density by *Micromeritics Accu Pyc 1330-Alphagas HE-200 Bara*, poured and tapped density by *J.Engelsman A.G Apparatebau* and Hausner Ratio was calculated. Residual moisture was measured by Mettler LP 16 Infrared drying unit and flowability by Erweka GT. The samples were further analysed on qualitative characteristics, such as superficial morphology by Scanning Electron Microscopy (SEM) using a Philips ESEM XL 30 FEG.

• *Preparation of tablets*

According to the two factor spherical second order Central Composite Design (CCD) ten formulations of tablets F_1-F_{10} were prepared The two screened factors % of *Carbopol 71G* (X_1) and and the crushing strength of the tablet (X_2) were varied -**table 1.**

Table 1: Model formulations of compressed matrix pellets

Pellets	X_1	Crushing strength of the tablet (N)	X_2	Carbopol®71G (%)
F_1	-1	40	-1	10
F_2	+1	80	-1	10
F_3	-1	40	+1	30
F_4	+1	80	+1	30
F_5	$-2^{1/2}$	31,7	0	20
F_6	$+2^{1/2}$	88,3	0	20
F_7	0	60	$-2^{1/2}$	5,86
F_8	0	60	$+2^{1/2}$	34,14
F_9	0	60	0	20
F_{10}	0	60	0	20

Table 2. Results for the pellets characterization

% Carbopol	Residual moisture [%]	Particle size distribution average [μm]	True density [g/ml]	HR	Flowability s/100g
10	2.20	645.00	1.4955	1.2048	16.20
30	2.94	754.61	1.4717	1.2306	18.83
20	3.44	796.30	1.4960	1.1684	20.30
5.86	2.98	628.40	1.4435	1.2399	11.83
34.14	2.11	509.83	1.4864	1.1354	19.50

The pellets with added Aerosil® and Magnesium stearate, were compressed into tablets on the Presster™ compaction simulator (Metropolitan Computing Corporation).The crushing strength of the tablet was controlled by adjusting the gap between the upper and lower punch. Flat faced punches of 7 mm in diameter were used and a rotary tablet press was simulated at the speed of 4000 tablets per hour. Matrix tablets were examined on weight, thickness, crushing strength and friability.

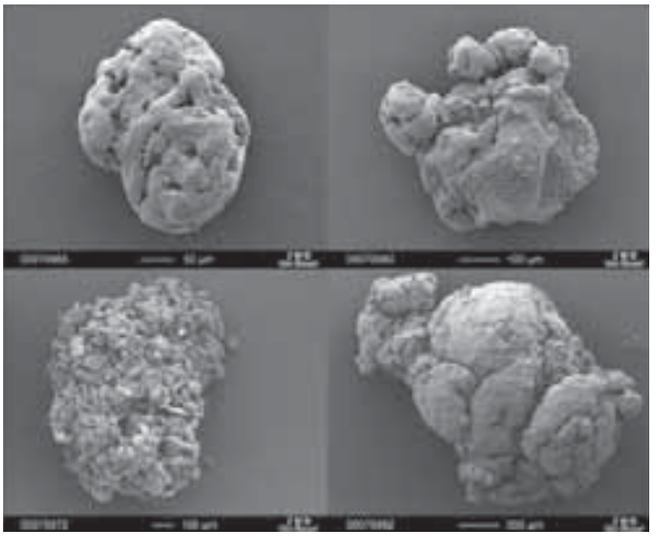

Figure 1: SEM pictures of pellets F_1, F_3 F_8,F_{10}

• Drug release study

Dissolution study was performed using Erweka dissolution tester DT-80 (pH 6.8, 900 ml for 8h at 37° ± 5°C) using the USP paddle apparatus at stirring speed of 50 rpm. The concentration of diclofenac sodium was determined by UV-VIS spectrophotometric method at 276 nm.

• Application of GRNN

GRNN was the optimization method that was used for development of diclofenac sodium compressed matrix tablets. Selected GRNN structure had four layers: first layer with two input units: different percent X_1 - *% Carbopol 71G* (5.86% - 34.14%) and X_2 – crushing strength of the tablet (31.7 – 88.3N),. Second layer of the network had 10 hidden units,. This units in hidden layer were assigned using *K means Centar Assignment* algorithm. A third layer had six units. Fourth layer had five output units-. (responses) which represented percent of released diclofenac sodium after 0.5, 1, 2, 4 and 8 hours.

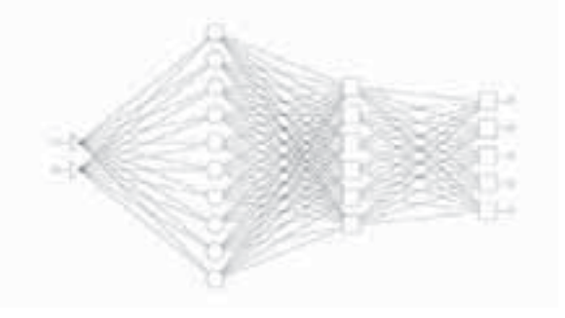

Figure 2: Architecture of GRNN applied in optimization

RESULTS AND DISCUSSION

Results of pellets characterization are presented in Table 2 and Figure 1. Having in mind that there are great differences in applied concentrations of Carbopol as matrix substance, obtained values of true densities,

as well as particle size distribution were very close.
Results of drug release studies indicated that drug release rates varied between different formulations, with a range of 1 hour to 8 hours of dissolution (Figure 3). GRNN was trained with drug release results from formulations F1-F10. Root mean square error for the network, reached after the training, was 0.04% which is an acceptable value.

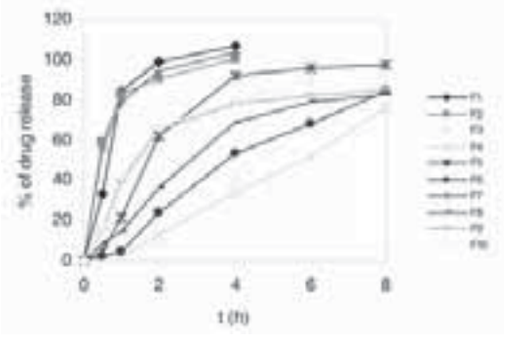

Figure 3: Dissolution profiles of compressed pellets F_1-F_{10}

Furthermore, in order to validate the prediction performance of the trained network, two additional formulations were prepared, with values of causal factors that differ from values that had been presented to the network. Drug release from these test formulations were analyzed. Obtained results showed that the GRNN was properly trained and capable to generalize problem.

• Optimization

Optimization was the final step in application of GRNN and it consideres calculation of optimal network input, percent of polymer and crushing strength which enables drug release according to requrements for diclofenac sodium extended release that were set up in the beginning of the experiment.. Increase of polymer resulted in decrease of percent of diclofenac sodium released after 8 hours. Influence of crushing strength of the tablet was less significant. This means that polymer % act is a controlling factor in the release of diclofenac sodium from the compressed matrix pellets. Optimal solution estimated with GRNN is formulation with 24 % Carbopol 71 G and 65 N of crushing strength. Optimal formulation was made and release profile obtained. Then, experimentally observed and GRNN predicted profiles were compared. Values f_1=6.93 and f_2=77.72 in this study indicate that drug release from matrix pellets were well optimized using GRNN.

CONCLUSION

Application of artificial neural network, in this case GRNN, as optimisation method completely realize the purpose of this study: optimal formulation of diclofenac sodium matrix pellets compressed into the tablets with Carbopol 71G as a matrix substance was achieved .

References

1. S.Ibric, M.Jovanovic, Z.Djuric, J.Parojcic, S.Petrovic, Lj. Solomun, Journal of Controlled Release 82 (2002) 213-222
2. M.Leane,I.Cumming, O.Corrigan AAPS Pharma SciTech 2003:4(2) Article 26
3. Bourquin J, Schmidt H, Van Hoogevest P, Leuenberger H., Pharm Dev Technol. 1997:2(2):95-109,111-121

OP022

Study of the effects of drugs on the structures of sucrose esters and the effects of solid-state interactions on drug release

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INTRODUCTION

Sugar esters (SEs) have a wide range of hydrophilic-lipophilic balance (HLB) values (1-16) and hence can be applied as surfactants, or as solubility or penetration enhancers [1]. In general, SEs are used in hot-melt technology [2, 3]. In this study, drug-SE products were prepared by melt technology and investigated the solid-state interactions by DSC, X-ray diffraction, contact angle measurement, temperature sweep and dissolution tests. Two non-steroidal anti-inflammatory agents were used as model drugs: meloxicam (ME) and diclofenac sodium (DS). ME is a poorly water-soluble material, while DS is slightly soluble in water, its solubility increasing as the pH rises. Three SEs were chosen for the preparation of the products: one with high HLB (P1670), one with moderate HLB (S970) and one with low HLB (B370).

We earlier studied the influence of thermal treatment of SEs without active agents on the structure [4]. The aim of the present work was to examine the effects of active agents on the structures of SEs and the effects of the drug-SE solid-state interactions on the drug release.

EXPERIMENTAL METHODS

• Sample preparation

Drug-SE physical mixtures (in a ratio of 1:1) were melted in a porcelain dish in an oven (Factory for Laboratory Equipment, Budapest, Hungary), with heating from 25 °C to 100 °C, and then cooled back to room temperature, pulverized in a mortar and sieved to 200 μm.

• Differencial scanning calorimetry (DSC)

DSC studies were performed with a DSC 821^e (Mettler-Toledo GmbH, Switzerland). Samples of 10 mg were heated in a sealed aluminium pan. The samples were heated from 25 °C to 300 °C at a heating rate of 10 °C min^{-1}.

• X-ray powder diffraction (XRPD)
XRPD profiles were taken with a Philips X-ray diffractometer (PW 1930 generator, PW 1820 goniometer). The measurement conditions were as follows: Cu K radiation (=0.15418 nm), 40 kV, 35 mA. The basal spacing (d_L) was calculated from the diffraction peaks by using the Bragg equation.

• Contact angle measurements
The contact angle of the solids was determined by means of the sessile drop technique, using the OCA 20 Optical Contact Angle Measuring System (Dataphysics, Filderstadt, Germany). Surface free energy and the polarity values were calculated from contact angle data.

• Temperature sweep tests
For these measurements, a PaarPhysica MCR101 type rheometer (Anton Paar GmbH, Graz, Austria) was used (in controlled rate mode), equipped with a cone-and-plate measuring system. During the measurements, the temperature of the samples was modulated from 25 °C to 40 °C with a heating rate of 1 °C min^{-1} while the resulting viscosity changes were recorded.

• Dissolution studies
For the dissolution tests, the ME-SE or DS-SE melted products were filled into hard gelatine capsules. The capsules contained 15 mg of ME and 15 mg of SE, or 50 mg of DS and 50 mg of SE.
The release of the model drugs was studied by using Pharmatest equipment (Hainburg, Germany), at a paddle speed of 100 rpm. 900 ml artificial enteric juice with a pH of 7.5 (± 0.05) at 37 °C (± 0.5 °C) was used. The drug contents of the samples were measured spectrophotometrically (λ_{ME} = 362 nm; λ_{DS}= 276 nm) (Unicam UV/Vis spectrophotometer). The dissolution experiments were conducted in triplicate.

RESULTS AND DISCUSSION

The *DSC results* show that after melting, the structures of the SEs broke down. On cooling, the structures were built up again, but rearranged to different degrees [4]. The comparisons revealed that the drug brought about considerable structural changes in the SEs, to different extents with the three SEs.

In agreement with the DSC examinations, the *X-ray examinations* revealed that the structures of the SEs were rearranged after melting, to the accompaniment of a decrease in the degree of crystallinity. The change was greater when a drug was present, especially in the case of lipophilic B370.

Table 1: Contact angles, surface free energies and polarities of the materials

Materials	θ_{water} [°]	γ^p [mN m^{-1}]	γ [mN m^{-1}]	Polarity [%]
P1670	18.49	42.73	70.10	60.96
S970	46.79	29.75	55.25	53.85
B370	89.81	5.99	36.08	16.60
ME	61.56	15.56	60.08	25.90
DS	16.8	35.48	78.67	45.10

The distribution of the drugs in the SE melt is influenced by *the polarities* of the initial materials. The results are presented in Table 1. The different HLB values are manifested in the various polarity values of the SEs, while the different wetting properties of the two drugs point to possible drug-SE interactions.

Temperature sweep test shows that P1670 (with a high HLB) gelled over 35 °C, while S970 (with a medium HLB value) displayed high viscosity even at room temperature. Lipophilic B370 has poor wetting properties in water, and its viscosity does not increase with increase of temperature. In the presence of ME the viscosities of both P1670 and S970 are almost as high as those without drug. On the other hand, the viscosities of both SEs decreased considerably in the presence of DS (Fig. 1). This interaction can be influenced to a large extent by the dissolution of DS.

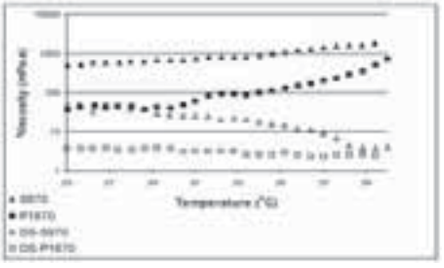

Figure 1: Viscosity of SEs and DS-SEs in water

The dissolution studies indicate that the rates of dissolution of the different drugs were influenced differently by the SEs. It was found that P1670 gelled at 37 °C, which explains why 100% release could not be achieved in the case of ME despite the high HLB value. DS was dissolved in the intestinal juice within a few minutes, and the effect of the hydrophilic P1670 was not manifested here. As the viscosity of P1670 was decreased considerably by the drug in aqueous medium, the dissolution of DS could not be delayed with this SE. In this case, the interaction between the drug and the SE plays a role, which is related partly to the different pH (pH of DS: 7.8; pH of P1670: 5.5) and partly to the salting-out effect of DS.

CONCLUSION

The DSC and XRPD results revealed that the structures of the SEs were rearranged, with a decrease in the degree of crystallinity. The dissolved drug molecules broke down the structures of the SEs, but were not build into the crystalline phase of the carrier. The rates of dissolution of the drugs were influenced by the different HLB values and gel-forming behaviour of the SEs, and also by the polarity of the drug and the interactions between the drug and the SEs.

ACKNOWLEDGEMENTS

Authors are grateful to Mitsubishi-Kagaku Foods Corporation (Japan) for providing sugar esters.

References

1. Mitsubishi- Kagaku Foods Corporation, Ryoto Sugar Ester Technical Information (1982)
2. M. Otsuka, T. Ofusa,, Y. Matsuda, Colloids Surf. B. 10, 217-226 (1998)
3. S. Marton, A. Auner, G. Csoka, Eur. J. Pharm. Sci. 25S1, S155-S157, (2005)
4. A. Szűts, E. Pallagi, G. Regdon jr., Z. Aigner, P. Szabó-Révész, Int. J. Pharm. 336, 199-207 (2007),

OP023

System aproach to finding surrogates for in-vivo studies of special dosage forms

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INTRODUCTION

This report aims to analyse influences of presystemic proceses, like the dosage form disintegration, drug dissolution (release) etc., on a given systemic drug performance measure, in this case the concentration-time profile. The method is based on the *in-silico* modeling supplemented by the sensitivity analysis. This enables judging whether the dissolution test waives *in vivo* studies ir not. Putting it into a more general setting, the drug formulator may be curious about the extent to which the modified presystemic processes may influence the concentration profile. In this regard the method is an extention of the traditionally conceived *in vitro - in vivo* correlation (IVIVC) analysis, which captures relationships between the *in vitro* dissolution and *in vivo* absorption profiles after administration of oral solid dosage forms.

A kind, structure, composition and manufacturing play dominant roles in the drug development and optimization. No wonder that the there has been a permanent endeavour to develop a similar tool for special dosage forms. Besides, there exists a groving need of more sopfisticated drug delivery systems, while securing their safety, patient compliance and low cost. Having this in mind, the paper presents an alternative to IVIVC, that may be apllicable to special dosage forms, (suppositories, chewable tablets and gums, transdermal patches, semisolid dosage forms, microparticles etc.) with the aim to avoid the cost and long lasting *in vivo* studies. The fact is that ground principles of developing and validating the IVIVC models of these systems are still unclear. [1,2] An IVIVC model can be easily developed if the *in vitro* dissolution is a rate-limiting stage of presystemic processes. But the complex relations between various dissolution profiles and the corresponding *in vivo* responses caused that scientists have been often contended with a certain degree of similarity between dissolution and absorption profiles, supposing (sometimes erroneously) that this similarity automatically produces the same similarity between corresponding *in - vivo* performances.

Similarities between dissolution profiles are routinely evaluated on the basis of the "difference factor f_1" or "similarity factor f_2" , which compare the profiles in a finite number of points only. [4]

USING PHARMACOKINETIC MODELLING IN THE IVIVC EVALUATION

In this approach the entire process, consisting of all pre & post absorption processes, is modelled and simulated by a computer. The tightness of the correlation between predsystemic processes and concentration prolile is predicted by using so called influence functions, expressing the strength to which the particular presystemic process influences the plasmatic concentration profile. Due to this, the corelation may be displayed continuously and in parallel to the progress of the drug distribution, rather then by single values of the f_1 or f_2 factors. That means a significant enrichment of the information the designer has on her/his disposal when designing a new or modifying an existing dosage form. In such a "holistic" approach all pre-systemic and systemic processes are conceived as a unique process, which if needed, may be further detailed so as to incorporate other subprocesses running inside the body, like the drug degradation, transport across biological membranes, the first pass effect etc. Having this in mind, one should admit that there are justified reasons why the mere similarity of the two dissolution profiles may not be sufficient. This is mainly true for the specific dosage forms. To demonstrate the basic principles, let us consider presystemic processes shown in Fig 1. Due to the variety of structures of the dosage form and the related presystemic mechanisms a plethora of various models of the presystemic processes may be built up. Hence, the main advantage of the presenteded approach w.r.t. the traditional IVIVC modelling and evaluation is that it directly relates the presystemic parameters to the drug perfomace. To this end some typical models of presystemic processes with both the instant and controlled release have been analysed and their influence on the plasmatic concentration were predicted on the basis of the influence analysis. [3] For instance, it was supposed in the experiment that the disintegration (liberation) follows the first-order linear kinetics, that the dissolved amount follows the Higuchi square-root law etc. Also the different disintegration, dissolution and absorption models were analysed. Because the different dissolution and absorption rates cause the different inflow and outflow of the drug to and from an absorption site, a balancing reservoir of the dissolved but not yet absorbed drug was inserted as shown in Fig. 2, where the blocks' dynamics are expressed by so called transfer functions. [3] Both the reservoir and distribution compartment were modelled by the first–order transfer functions as shown in Fig. 2

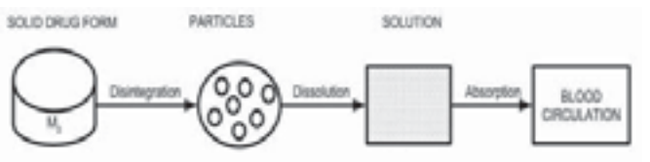

Figure 1: Basic presystemic processes

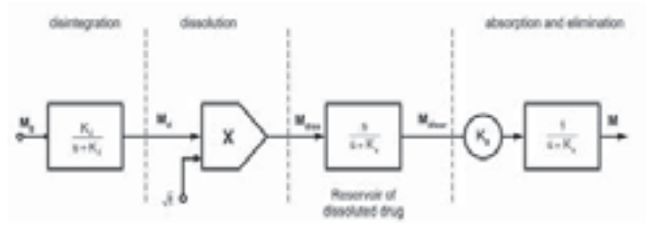

Figure 2: Models of predistribution processses

RESULTS AND DISCUSSION

Both the presystemic and systemic processes were modelled in accordance with the principles of the pharmacokinetic modelling and influences of presystemic processes on the concentration profile were evaluated by using the influence (or sensitivity) analysis, which is not presented here. The results obtained are summarized in Figs 3 and 4. The figures display sensitivities S of the drug amount M in the blood circulation for two different cases: the instataenous (or at least very quick) and slow disintegration respectively. The quick disintegration leads to the smaller senstitivity of M to the modifications of the disintegration process (disintegration rate constant K_{diss}) and vice versa. The similar conclusions may be drawn for the dissolution. The results vere validated by an *in vivo* experiment. Therefore, for a (special) dosage form with the quick disintegration and/or dissolution, the dissolution test may be qualified as a waiver of the *in vivo* studies. In other words, the lower sensitivity to presystemic processes the stronger possibility to waive *in vivo* studies. Hence, the systems model - based experiment may be made into an effective surrogate for *in vivo* studies.

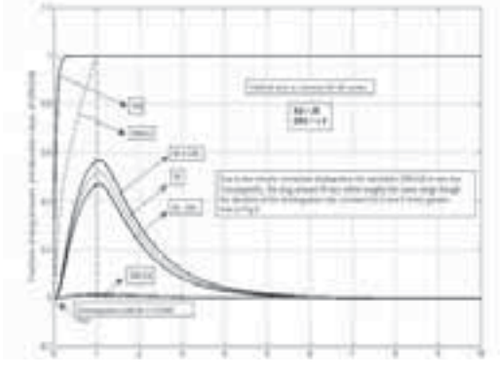

Figure 3: The case of a very quick disintegration

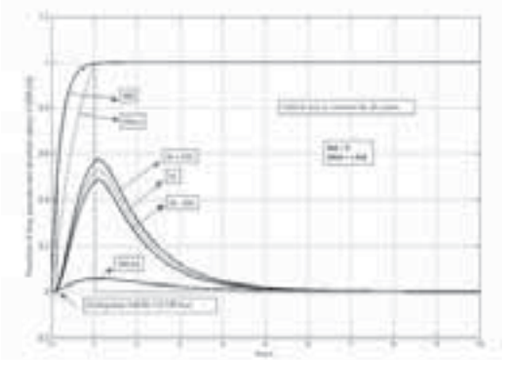

Figure 4: The case of a slow disintegration

ACKNOWLEDGEMENTS

Grants VEGA No.1/0320/08 and No.3120 of the Slovak Ministry of Education are duly acknovledged.

References

1. Sunkara G., Chilukuri D. M. IVIVC: An Important Tool in the Development of Drug Delivery Systems. Drug Delivery, 3: 234 -242 (2003).
2. Kalász H., Antal I. Drug Excipients. Current Medical Chemistry. 13: 2535 - 2563 (2006).
3. Rosensenwasser E., Yusupov R. Sensitivity of Automatic Control Systems. CRC Press, London, 2000.
4. Moore, J.W., Flanner, H. H. Mathematical Comparison of Curves with an Emphasis on Dissolution Profiles. Pharmaceutical Technology. 20: 64-74 (1996).

OP024

Development and evaluation of new solid state microemulsion based drug delivery system

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INTRODUCTION

Development of new drug delivery systems with the intention of enhancing the efficacy of existing drugs is an ongoing process in pharmaceutical science. Several drugs demonstrate low bioavailability because of poor dissolution in physiological fluids as well as poor permeability. Use of microemulsion based systems (microemulsions and self microemulsifying drug delivery systems (SMEDDS)) may enhance oral bioavailability by increasing the solubility of poorly water soluble compounds. Because liquid dosage form is problematic regarding stability and dosing, many attempts for conversion of these liquid systems into solid state are described in literature such as adsorption and drying [1].

Classical dissolution tests containing only buffer solution are not suitable for determination of bioavailability of poorly water soluble drugs because endogenous bile salts and phospholipids facilitate drug dissolution in gastro intestinal tract (GIT). Use of similar surface active agents in dissolution medium is essential for better prediction of drug behavior

in GIT. The mechanism of absorption increase of poorly water soluble drug which is incorporated in ME is not fully understood, however some findings show that drug incorporates into mixed micelles which facilitate drug transfer through intestine wall [2]. The effect of higher dissolution on bioavailability could be determined using in-vitro models such as side-by-side diffusion chambers with rat jejunal segments.

A model poorly water soluble drug was incorporated into powder containing solid state SMEDDS. Two different formulations were tested. Obtained results show higher dissolution of drug from our new solid microemulsion based drug delivery system in comparison with commercially available tablets containing same active ingredient.

EXPERIMENTAL METHODS

Solid state SMEDDS were prepared as follows. Povidone as solid carrier was dissolved in water and this solution was mixed with already prepared SMEDDS composed of polysorbate 80, glycerol monocaprylate (Imwitor 308), medium chain triglyceride (MCT). This dispersion was spray dryed in laboratory scale spray dryer (Air-pro, Pro-Cept, Belgium). As a model drug poorly water soluble candesartan cilexetil (CC) with solubility less than 1,0 μg/ml, was incorporated. Spray dried powder was granulated with ethanol and different excipients were added. The granulate was compressed into 400 mg tablets using laboratory rotary tablet press. Two different formulations which differ in dissolution profiles and are shown in Table 1 were made.

Table 1: Composition of solid ME based drug delivery system.

	Formulation I [mg]	Formulation II [mg]
Medium chain TGC	13,4	13,4
Polysorbate 80	40,2	40,2
Imwitor 308	13,4	13,4
Povidon	101,0	101,0
Candesartan clx (CC)	32,0	32,0
MCC	76,0	
Crospovidone	120,0	
Manitol		98,0
Soribitol		98,0
Mg. Stearat	2,0	2,0
Aerosil	2,0	2,0
Total	400,0	400,0

The dissolution characteristics of formulations were tested at 37°C according to USP, apparatus 2 with 900 ml of media volume and at 75 RPM. Two media were used: 50 mM KH_2PO_4 buffer and simulated intestinal fluid - SIF (50 mM KH_2PO_4, 2,5 mM sodium deoxycholate and 0,5 mM lechitin). Concentration of dissolved drug was determined using UV spectrophotometer.

The transport experiments across excised rat jejunal segments were performed in side-by-side diffusion chambers under the short circuit conditions. During the experiments the tissue was bathed on both sides with Ringer buffer (pH=6.85, 37°C) containing 10mM D-glucose at the serosal and 10 mM mannitol at the mucosal side and gassed with carbogen. The viability of the tissue was monitored throughout the experiment by recording the transepithelial potential difference, transepithelial electrical resistance and short-circuit current. Tablets were disintegrated in simulated intestinal juice and 3 ml of dispersion was applied on donor side of diffusion cells. The apparent permeability coefficients (P_{APP}) of fluorescein were calculated according to the equation: $P_{APP}=(dQ/dt)/(A \cdot C_0)$ (cm/s), where dQ/dt is the steady state appearance rate of the tested substance on the acceptor side of the tissue, A is the exposed area of the tissue (1cm^2), C_0 is the initial concentration of the tested substance in the donor compartment. The concentrations of candesartan cilexetil and its metabolite in the samples taken from the acceptor and donor compartment were analysed by HPLC.

RESULTS AND DISSCUSSION

By using different final tablet excipients we were able to achieve different dissolution profiles for formulations I and II. The release of CC from both SMEDDS tablets is greater compared to the reference (Atacand) formulation. However, this difference is smaller, although significant, in SIF (Fig.2) compared to the simple phosphate buffer medium (Fig.1), most probably as a result of the presence of surfactants in the SIF.

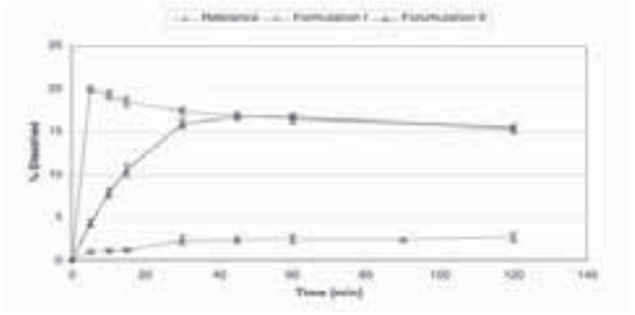

Figure 1: Dissolution of candesartan cilexeril in 50 mM phosphate buffer.

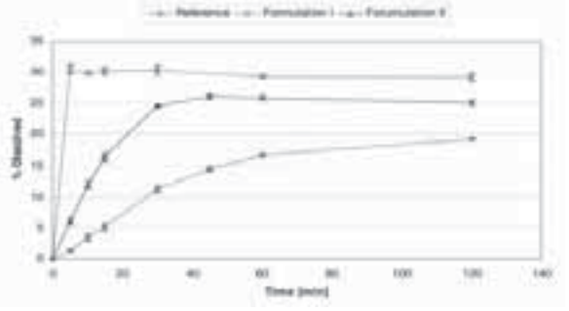

Figure 2: Dissolution of candesartan cilexetil in simulated intestinal fluid.

As shown from Figure 3, candesartan cilexetil at the mucosal side was rapidly metabolized to its metabolite, candesartan. Only candesartan was present at the serosal side, demonstrating a complete metabolism during the absorption across the intestinal epithelium. Although the concentrations of CC and its metabolite at the mucosal side were higher in case of formulation II compared to the reference formulation, no significant differences in the permeated amounts were observed as a result of decreased P_{APP} (P_{APP} values were 3.9 $10^{-5} \pm 0.4$ 10^{-5} cm/s and 1.0 $10^{-5} \pm 0.1 \pm 10^{-5}$ cm/s for the reference and formulation II, respectively). Lower P_{APP} in case of formulation II might be explained by incorporation of CC in the micelles formed by polysorbate 80, thus decreasing the diffusion coefficient.

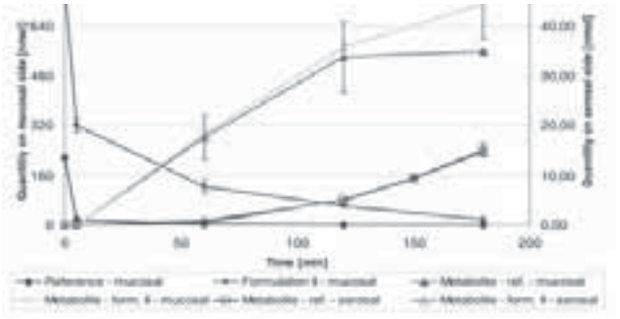

Figure 3: Amount of CC and its metabolite on mucosal and serosal side from reference and our formulation II.

CONCLUSION

The results of this study demonstrate the enhanced dissolution of CC from the solid state ME based delivery systems compared to the marketed formulation. Although, the results using excised rat jejunal segments show decreased permeability of CC for solid state ME based delivery systems, further in vivo studies are needed to evaluate the absorption processes of this new delivery system.

References

1. Christensen K L, Pedersen G P, Kristetnsen H G: Preparation of redispersible dry emulsion by spray drying. Inernatiolnal Jurnal of Pharmaceutics 2001; 212: 187-194.
2. Porter C J H, Charman W N: In vitro assessment of oral lipid based formulation. Advanced Drug Delivery Rewievs 2001; 50: 127-147.

OP025

Density functional theory calculations on meloxicam-β-cyclodekstrin inclusion complexes

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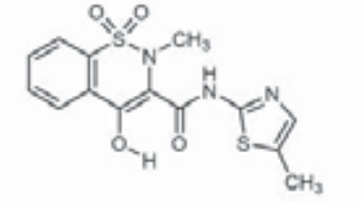
INTRODUCTION

Meloxicam (MEL) is a highly potent non-steroidal anti-inflammatory drug (NSAID) of the enolic acid class of oxicam derivates, which exhibits in animal tests a high potency for potential anti-arthritic action and a wide spectrum of anti-inflammatory activities, combined with less gastric and local tissue irritation than other NSAIDs. It is barely soluble in water.
The complexation with β-cyclodextrin (β-CD) leads very often to a significant increase of the solubility and, consequently of the bioavailabilty of many drugs. It serves also as an effective drug delivery system for several NSAIDs, and therefore it can be used for solubility enhancement of MEL. Experimental investigations on the solubility of MEL at various pH-values in the presence of β-CD have proven the significant enhancement of the solubility of MEL and the change of the dissolution rate of the drug attributed by the host guest association - by the inclusion of the drug into the CDs cavity [1].



Meloxicam (MEL) is a two-basic acid with a series of tautomers in each protonation state, which are able to form various intramolecular hydrogen bonds. In aqueous solution also different intermolecular hydrogen bonds can be formed, and under physiological conditions (aqueous solution, pH 7.4) the anionic form of meloxicam is the predominant tautomer. Generally, structures with a higher dipole moments, like zwitterionic forms, are more stabilized in aqueous solution than in the more hydrophobic environment. Therefore, the tautomeric equilibria as well as the protonation and deprotonation steps are shifted by the inclusion reaction with β-CD. The experimentally observed overall equilibrium constants consist of the individual equilibrium constants of those conformers which exist at thermodynamic equilibrium.
In the present study the tautomers of MEL, particularly in the unprotonated state are investigated by molecular modelling methods in gas phase and the geometries of the complexes with β-CD are analysed.

METHODS OF CALCULATION

DFT studies at the B3LYP/6-31G(d,p) level have been used to calculate the geometries and the energies of formation of the inclusion complexes of β-CD with neutral, deprotonated and protonated form of MEL without taking into account the influence of the solvent. The affinities of the individual tautomers and conformers to CDs can be estimated from the energy differences between the calculated complexes and the isolated molecules.
The inclusion complexes were constructed by moving the guest (in two different orientations) along the z-axis perpendicular to the plane of the cyclodextrin linkage oxygens, entering the host at the wider rim with the benzene ring first. All complexes were then fully geometry optimized.

RESULTS AND DISCUSSION

The calculations show that in all cases the molecule is located inside the cavity. Both orientations are energetically possible. The preferred com-

plexation orientation is that one, in which the benzene ring of MEL is located near the wider rim with the secondary hydroxyl groups of the CD.

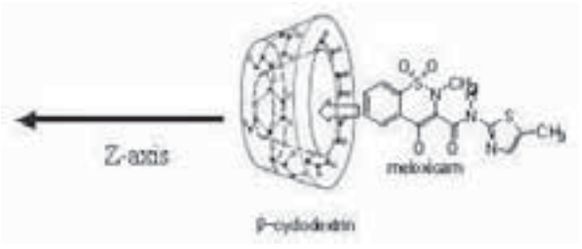


Figure 1: Orientation of MEL in the β-CD/MEL complex.

In Table 1 the calculated interaction energies are given. These formation energies for the encapsulation of the meloxicam guest molecules show an overall affinity ranking for the MEL guest molecule in the following order: Deprotonated form > neutral form ~ zwitter ionic form > protonated form. The stabilization of the complexes is, as expected, influenced by hydrogen bonding. Nevertheless, the calculations are performed on molecules in gas phase, neglecting the fact that complex formation results from differences in solvation energies of the host and the guest compared with the solvation energies of the host-guest complex system. So, therefore, the obtained energies are interaction energies of the isolated molecules without solvation energies and the entropic contributions.

Table 1: Comparison of the calculated inter-action energies (B3LYP/6-31G(d,p) in kcal/mol) of the con-formational minima of various meloxicam tautomers complexed with β-CD.

Complex	Form	ΔE of the complex (in kcal/mol)
β-CD + MEL P	1	-3.32
β-CD + MEL P	2	-9.90
β-CD + MEL N	1	-25.84
β-CD + MEL N	2	-28.88
β-CD + MEL Z	1	-3.62
β-CD + MEL Z	2	-5.63
β-CD + MEL D	1	-8.92
β-CD + MEL D	2	-10.55

CONCLUSION

The application of B3LYP/6-31G(d,p) on CD inclusion complexes leads to more reliable geometries than the widely used semiempirical methods. The results show that the MEL can be completely inserted into the β-CDs cavity in two different orientations. The largest interaction energies are observed for the anionic form of MEL.

References

1. Charumanee S., Titwan A., Sirithunyalug J., Weiss-Greiler P., Wolschann P., Viernstein H., Okonogi S. Thermodynamics of the encapsulation by cyclodextrins. J. Chem.Technol. Biotechnol. 81: 523-529 (2006).

PO001

Chitosan-hyaluronic acid nanoparticles loaded with heparin for the treatment of asthma

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INTRODUCTION

A component of tissue remodeling and narrowing in asthmatic airways involves the accumulation of airway smooth muscle (ASM). This factor is suggested to cause airways hyperresponsiveness (AHR) [1]. Although mast cells produce mediators that interact with ASM cells to cause AHR [2], they are the only source of heparin in mammals and have a protective role by limiting inflammation and airway remodeling [3]. Additionally, several works had demonstrated that inhaled high, medium, and low-molecular-weight-heparin were effective in preventing the bronchoconstriction and the AHR. This effect was attributed to the prevention of mastocites-degranulation [4].

Considering the high net negative charge of heparin, the high-molecular-weight of the different heparins, and the presence in the airways of mucociliary and enzymatic activity, it is possible to anticipate low probabilities that heparin, administered topically, could prevent the accumulation of ASM in the airways. A possibility to overcome these problems is the use of nanocarriers that are capable of positively interacting with the pulmonary mucosa and transport the associated heparin to the ASM, thereby also preventing the enzymatic attack.

Chitosan (CS) is a natural, polycationic, and mucoadhesive polymer that has demonstrated to be able to prolong the residence time of drug delivery systems at the site of absorption [5], and additionally, to open transiently the tight junctions of epithelial cells [6]. Hyaluronic acid (HA) is another interesting polymer because is biocompatible, mucoadhesive, and exist a variety of receptors for HA (called CD-44) present in the surface of a variety of cells that could improve the interaction between the polymer and target cells [7].

The aim of this study was to combine the properties of CS with those of HA to prepare nanosystems loaded with two different molecular weight heparins. In the future, these nanoparticles will be testing for the asthma therapy.

EXPERIMENTAL METHODS:

Materials: CS hydrochloride salt deacetylated in 85% (UPCL113, Pronova), sodium hyaluronate (2/0003, bioiberica). Unfractioned heparin (UFH), low-molecular-weight-heparin (LMWH), and tripolyphosphate (TPP) were purchased from Sigma.

Nanoparticles preparation: Nanoparticles were prepared using the ionotropic gelation method [8]; briefly 3.5 mL of an aqueous mixture containing HA, TPP and UFH or LMWH were added to 3.5 mL of a CS solution under magnetic stirring at room temperature. The nanoparticles were isolated by centrifugation in a glycerol bed (16000 *g*, 30 min, 25 °C). Supernatants were collected for determinate the amount of unbound heparin using a colorimetric method (Stachrom® Heparin, Diagnostica Stago). Nanoparticles were then resuspended in ultrapure water by shaking using a vortex. **Nanoparticles characterization:** Size and ζ potential were determined by photon correlation spectroscopy and laser Doppler anemometry (Zetaziser® 3000 HS, Malvern); morphology by TEM (CM12, Phillips); stability by the evolution of the size under incubation at 37°C in different media; and the release by the measurement of heparin released in PBS pH 7.4 at 37°C.

RESULTS AND DISCUSSION:

In the case of nanoparticles made with CS-HA-TPP loaded with UFH, it was necessary to find the suitable ratio of the components at which the nanosystems were formed and could be adequately isolated (see table 1). It is possible to argue that when the amount of polyanions was too low, nanoparticles were not formed. When the amount of polyanions was increased, nanoparticles with different characteristics were obtained, but if the amount of polyanions was too high, the nanosystems were irresuspendable or precipitation occurred. Additionally, when the amount of polyanions was increased, discreet diminutions in zeta potential were observed. This situation might be caused by an increased shielding of free positively groups of CS. Among the formed nanosystems we selected one of them (**in bold**) and exchange the UFH by LMWH.

Table 1: Physicochemical characteristics of the CS-HA-TPP nanoparticles loaded with UFH or LMWH (n=3).*

Amount (mg) CS-HA-TPP-UFH or LMWH*	Size (nm)	PDI	ζ potential (mV)
4-1.2-0.21-1	201 24	0.2–0.4	+32 2
4-1.2-0.21-1.2	217 30	0.2–0.4	+28 1
4-1.2-0.21-1.4	Irresusp.	---	---
4-1.2-0.21-1.6	Precipit.	---	---
4-0.6-0.21-1.2	162 17	0.1–0.3	+35 1
4-0.6-0.21-1.4	**193 32**	**0.2–0.4**	**+33 2**
4-0.6-0.21-1.5	Irresusp.	---	---
4-0.6-0.21-1.4*	**152 10**	**0.2-0.3**	**+33 1**

All formed systems were in the nanosized range and showed positive zeta potential (Table 1). Loading, association efficiency, and yield of the selected formulations are shown in Table 2.

Table 2: Loading capacity, association efficiency and yield of the selected nanoparticles (n=3).

Amount (mg) CS-HA-TPP-UFH or LMWH*	Loading (%)	Ass. eff. (%)	Yield (%)
4-0.6-0.21-1.4	**34 ± 1**	**72 ± 3**	**49 ± 1**
4-0.6-0.21-1.4*	**61 ± 1**	**70 ± 8**	**25 ± 4**

TEM micrographs show the spherical morphology of the selected nanoparticles with HNF or LMWH (see figures 1A and 1B, respectively)

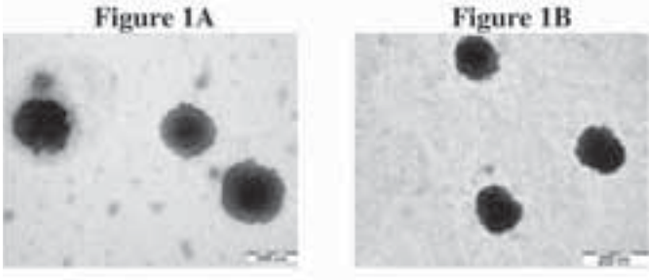

Figure 2 indicates that in PBS pH 7.4, both nanoparticles-formulations were stable, whereas in HBSS pH 6.4, the formulations gradually increase in size being finally destabilized.

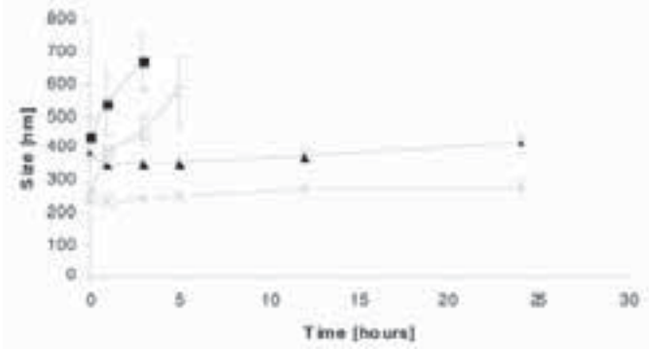

Figure 2: Stability of selected nanosystems at 37 °C, loaded with: UFH in PBS pH 7.4 (▲), LMWH in PBS pH 7.4 (x), UFH in HBSS pH 6.4 (■), and LMWH in HBSS pH 6.4 (□) (n=3).

Figure 3 shows that the release of UFH and LMWH from the selected nanosystems in PBS pH 7.4 occurred slowly without showing any burst effect.

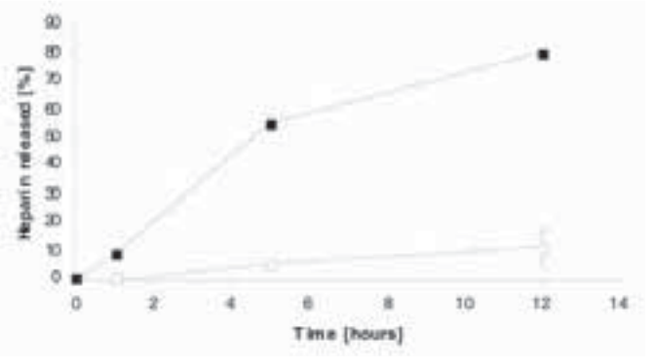

Figure 3: Heparin release profiles of selected nanoparticles in PBS pH 7.4 at 37 °C. UFH (■), and LMWH (□) (n=3).

CONCLUSIONS:

Considering the beneficial properties that combinations of CS with HA could offer to the pulmonary administration of heparin as antiasthmatic drug, CS-HA nanoparticles loaded with this drug have been prepared and characterized. Selected nanosystems showed desirable characteristics in terms of stability and in vitro heparin release, being promising for preventing the hyperproliferation of ASM, the mastocites-degranulation and probably for others applications in the treatment of asthma.

ACKNOWLEDGMENTS:

The authors acknowledge to the Spanish Government for the financial support (SAF 2004-08319-C02-01 and Consolider-Ingenio CSD 2006-00012), and to CONICYT for the scholarship of Felipe Oyarzun-Ampuero.

References:

1. James, A.L., Pare, P.D., Hogg, J.C., 1989. Am. Rev. Respir. Dis., 139, 242-246.
2. Robinson, D.S., 2004. J. Allergy Clin. Immunol., 114, 58-65.
3. Page, C.P., 1991. Lancet, 337, 717-720.
4. Molinari, J., Campo, C., Shakir., Ahmed, T., 1998. Am. J. Respir. Crit. Care Med., 157, 887-893.
5. Soane, R.J., Frier, M., Perkins, A.C., Jones, N.S., Davis, S.S., Illum, L., 1999. Int. J. Pharm., 178, 55-65.
6. Garcia-Fuentes, M., Prego, C., Torres, D., Alonso, M.J., 2005. Eur. J. Pharm. Sci., 25(1), 133-143.
7. Jaracz, S., Chen, J., Kuznetsova, LV., Ojima, I., 2005. Bioorg. Med. Chem., 13, 5043-54.
8. Calvo, P., Remuñan-Lopez, C., Vila-Jato, J.L., Alonso, M.J., 1997. J. Appl. Pol. Sci., 63, 125-132.

PO002

Mixed poloxamer/chitosan systems as nanocarriers for dexamethasone

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INTRODUCTION

Poor ocular bioavailability is the result of the fast elimination of the applied drug and relative impermeability of the corneal epithelial membrane. Drug delivery systems could help to overcome these problems by providing prolonged contact with the eye surface, sustained release of drug and enhanced corneal epithelial permeability. Poloxamers are the block copolymers consisting of poly(oxyethylene) and poly(oxypropylene) units (PEO/PPO/PEO). Due to inherent surface active properties, they were employed as solubilizers and proposed as artificial tears. In addition their possible enhancer effect on drug permeation through cell membranes has been reported. Among other polymeric carriers used, cationic chitosan has attracted considerable attention due to its biocompatibility, biodegradability and ability to enhance paracellular transport of drugs (1-2). Therefore, chitosan seems worthwhile exploring as permeation coenhancer in mixed poloxamer/chitosan colloidal nanosystem.

The aim of this study was to investigate the influence of chitosan on the stability, loading capacity and release of dexamethasone (DEX) from mixed poloxamer/chitosan systems.

MATERIALS AND METHODS

The poloxamer 407, (Lutrol® F127; molecular weight 12 600, BASF), low-viscous chitosan (molecular weight 150 000, deacetylation degree 84.5 %, Fluka) and DEX (JGL) were used as received.

Lutrol® F127 copolymer (F127) and mixed F127/chitosan systems loaded with DEX were prepared by an oil-in-water (O/W) emulsion/solvent evaporation method (3). DEX (10 mg) was dissolved in acetone (0.75 ml) and mixed with chloroform (0.75 ml) to form the oil phase. This oil phase was added dropwise with gentle stirring to an aqueous solution (10 ml) of F127 (5%, w/v) with or without chitosan (0.005 or 0.01 or 0.015%, w/v). After the organic solvent evaporation, the unincorporated DEX aggregates were removed by filtration through 0.20 μm filters (Milipore®). The DEX content was determined spectrophotometrically at 239 nm after the disruption of the micelles and the solubilization of DEX in ethanol.

The release of DEX was examined by a dialysis method. The sample (2 ml) was poured into a dialysis membrane (Dialysis Tubing Visking; M_w cut-off = 3500 Da) and subsequently incubated in 40 ml of SDS solution (0.03%, wt %) at 25°C with gentle stirring. At regular time intervals, aliquots (2 ml) were taken from the release medium, which was then replenished. The amount of DEX released was determined spectrophotometrically at 239 nm.

Hydrodynamic diameter (d_h) and zeta potential (ζ) of F127 and mixed F127/chitosan systems loaded with DEX were measured by photon correlation spectroscopy and laser Doppler anemometry, respectively (Zetasizer 3000 HSA, Malvern Instruments). The samples were filtered through 0.20 μm membrane filter (Milipore®) before measurements. The measurements were preformed at 25°C.

RESULTS AND DISCUSSION

The main characteristics of mixed F127/chitosan nanosystems prepared in this study are presented in Table 1.

Table 1: Characteristic parameters of F127 and mixed F127/chitosan systems loaded with DEX.

Sample	d_h (nm)	PDI	ζ (mV)
F127	28.6 ± 0.7	0.538	-2.1 ± 0.3
F127/0.005 w/v% CH	27.4 ± 0.9	0.554	14.4 ± 0.8
F127/0.01 w/v% CH	29.2 ± 0.3	0.542	27.9 ± 1.2
F127/0.015 w/v% CH	29.2 ± 0.9	0.567	31.9 ± 1.5

* CH = chitosan; PDI=polydispersity index, Values are mean SD (*n*=3)

Surface charge of nanosystems switched from almost neutral to highly positive with the chitosan presence and increased proportionally to chitosan content (Table 1). Results obtained for hydrodynamic diameter, polydispersity index and DEX loading indicate that chitosan inducement in the F127 system did not influence the micelle formation, their size, drug loading capacity and stability (Table 1 and 2).

Table 2: Entrapment efficiency (EE) and drug loading (DL) of F127 and mixed F127/chitosan systems loaded with DEX.

Sample	EE (%)	DL (%)
F127	26.11	0.52
F127/0.005 w/v% CH	24.11	0.48
F127/0.01 w/v% CH	24.21	0.48
F127/0.015 w/v% CH	24.26	0.48

The in vitro release profiles of DEX from F127 or mixed F127/chitosan nanosystems are presented in Fig. 1. All nanosystems are characterized by similar release profiles including an initial burst, followed by a slow release phase. This results indicate that chitosan did not impair the stability of the DEX-loaded micelles.

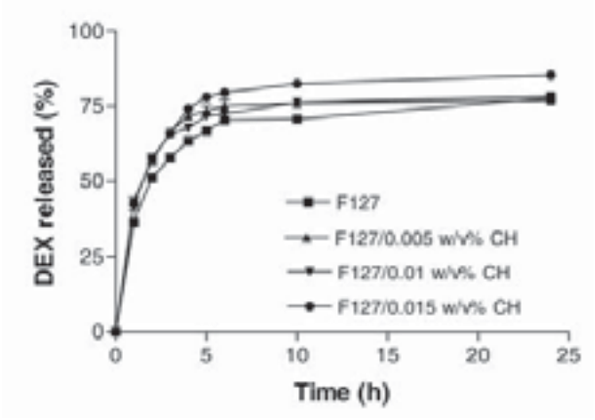

Figure 1: Cumulative release profile of DEX from F127 and mixed F127/chitosan nanosystems under sink conditions at 25 °C (mean ±SEM, n = 3).

CONCLUSION

Mixed poloxamer/chitosan systems as nanocarriers for dexamethasone have been successfully prepared. Chitosan inducement in the F127 system did not influence the micelle formation, their size, stability, drug loading and release. However, the systems developed were characterised with positive surface charge originated from chitosan thus showing potential for improved ocular delivery.

References

1. Liaw, J., Chang, S-F., Hsiao, F-C. (2001) In vivo gene delivery into ocular tissues by eye drops of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) polymeric micelles, *Gene Therapy*, **8**, 999-1004.
2. Maestrelli, F., Zerrouk, N., Chemtob, C., Mura, P. (2004) Influence of chitosan and its glutamate and hydrochloride salts on naproxen dissolution and permeation across Caco-2-cells, *International Journal of Pharmaceutics*, **271**, 257-267.
3. Kim, D-H. and Martin, D.C. (2006) Sustained release of dexamethasone from hydrophilic matrices using PLGA nanoparticles for neural drug delivery, *Biomaterials*, **27**, 3031-3037.

PO032

Evaluation of the properties of poly(lactide-co-glycolide) nanoparticles coated with bioadhesive polymers

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INTRODUCTION

An effective approach to improve the ocular availability of topically applied drugs is to insert them in polymeric nanoparticles [1]. Various properties of nanoparticles as their size, charge and hydration may influence both the residence time and the penetration of drugs through the cornea. The formation of a coating layer around particles is a promising approach to prolong their residence precorneal time. The use of bioadhesive polymers as coating agents could prolong the residence time because of the interaction with the ocular mucus layer.

The aim of the present study is to prepare poly(lactide-co-glycolide) nanoparticles providing longer residence time and consequently better drug bioavailability after local ocular application. The coating of nanoparticles with a bioadhesive polymer was suggested to enable this process. Two coating agents were applied, in particular chitosan and sodium alginate, because of their bioadhesive properties. Their concentrations were adjusted to give an equal viscosity of the coating phases aiming to investigate the effect of a certain polymer on the nanoparticle characteristics.

METHODS

Preparation of nanoparticles

An aqueous solution of pilocarpine hydrochloride (2.5 % w/v) was emulsified by sonication in 10 ml of a methylene chloride, containing 1.0 g poly(lactide-co-glycolide) copolymer (PLGA, Mw 40 000). The resulting emulsion was subsequently added to 50 ml of outer aqueous phase containing chitosan (0.1 or 0.5% w/v) or sodium alginate (0.007 or 0.118% w/v), and sonicated for 30 s. This w/o/w emulsion was then subjected to a high pressure (500 bar) using a microfluidizer (M-110L, Microfluidics, Newton, USA) and treated three cycles. The homogenized emulsion was placed in a distilled water phase and stirred at 700 rpm for evaporation of the organic solvent. The resulting suspensions were cooled down at –18°C and then freeze dried.

In parallel, non-coated nanoparticles were prepared by following the same procedure, but in the presence only of PVA in the aqueous phase of the multiple emulsion.

Determination of the size and surface charge of nanoparticles

Nanoparticle size was determined by photon correlation spectroscopy with a Zetasizer 3000 (Malvern Instruments, Malvern, UK). The freeze dried samples were dispersed in distilled water before measurements. Surface charge of the nanoparticles was examined by laser doppler anemometry using a Zetasizer 3000 apparatus. Measurements were performed in distilled water as well as in 0.1 % (w/w) aqueous mucin dispersion.

In vitro drug release studies

In vitro release studies were performed using vertical diffusion cell. The nanoparticles (20 mg) were placed as an aqueous suspension in the donor compartment of the cell, and the acceptor compartment was filled with distilled water. The concentration of the released pilocarpine was determined by HPLC method [2].

Turbidimetric measurements

Turbidimetric measurements were performed by means of a Hitachi U-1500 spectrophotometer at 650 nm (Hitachi Instruments Inc., Japan). The accurately weighed nanoparticles (10 mg) were added to 10 ml aqueous mucin dispersion (0.1% w/w) and stirred at 200 rpm. The turbidity of the dispersions was measured at certain time intervals and compared to the turbidity of the native mucin dispersion. The behaviour of nanoparticles was also examined in a mucin free aqueous dispersion under the same conditions.

Statistical analysis

Statistical evaluation was done by means of an ANOVA analysis, followed by a post hoc Newman Keuls test.

RESULTS AND DISCUSSION

Physico-chemical characteristics of nanoparticles

The particles formulated with chitosan were characterised with a statistically significant larger size comparing to the other nanoparticle series (Fig. 1). At the higher chitosan concentration in the coating phase (0.5 %), larger diameter of the resulted particles was observed. Similar phenomenon occurred using two concentrations of sodium alginate. However, chitosan-coated nanoparticles were larger than those modified with sodium alginate. The later could be due to the higher thickness of the resulted chitosan coating layer. Moreover, the surface charge only of chitosan coated particles was positive compared to the charge of the other nanoparticle types (non-coated and sodium alginate-coated). Thus, the larger size and the change of the surface charge from negative to positive values pressumed formation of the chitosan surface layer around particles.

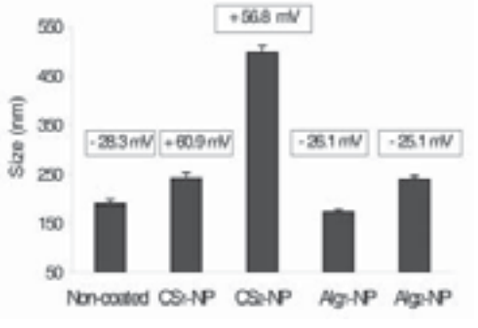

Figure 1: Influence of the coating on the size and surface charge (in boxes) of the nanoparticles.

In vitro release studies

The release process consisted of two phases, in particular initial burst phase that was followed by slower release thereafter. Significant burst effect was observed for non-coated particles, where 63 % pilocarpine was released for 3 hours. On the contrary, the coated nanoparticles demonstrated lower burst between 22 and 35 %. The high burst effect is often due to the high level of drug loading in the particles. However, this was not the main reason for the faster initial release from non-coated particles because the burst release from sodium alginate nanoparticles was slower independent on their higher drug loading (28% and 35%, respectively). In this case, the electrostatic attractions between pilocarpine hydrochloride and sodium alginate could additionally slow down drug release. Thus, the lower burst release for the modified nanoparticles could be considered as a consequence of the coating layer.

Evaluation of nanoparticles/mucin dispersions

Turbidity of nanoparticles/mucin aqueous dispersions was examined aiming to obtain preliminary information about the bioadhesive properties of the nanoparticles prepared. The turbidity of chitosan particles/mucin dispersions was higher either than the turbidity of mucin itself or non-coated particles/mucin dispersion.

To get insight in the mechanism of chitosan coated nanoparticles/mucin interaction zeta-potential measurements of their dispersions were performed. Eventual changes in their zeta-potential values during incubation with mucin could be considered as indication for their electrostatic interaction. Indeed, the positive charge of both nanoparticle formulations drastically dropped after incubation in mucin dispersion (Fig. 2). The surface charge of CS_1-coated-NP became even negative after incubation with mucin. The charge of the particles formulated with the higher chitosan concentration (CS_2-NP) also reduced, but even after 6 hours incubation it was still positive. These data correlated with the results from turbidimetric study. The turbidity of the same dispersion increased after 5 hours suggesting that the interaction between particles and mucin still continued.

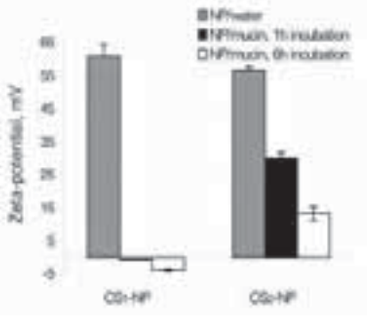

Figure 2: Estimation of the zeta-potential values of the nanoparticles during incubation in 0.1% mucin dispersion. The charge of nanoparticles in water was used as a reference.

CONCLUSION

The coating with chitosan could be a useful approach aiming to provide longer residence time of the nanoparticles after local ocular application.

References

1. Zimmer A., Kreuter J. Microspheres and nanoparticles used in ocular delivery systems. Adv. Drug Deliv. Rev. 16: 61-73 (1995).
2. Yoncheva K., Vandervoort J., Ludwig A. Influence of process parameters of high-pressure emulsification method on the properties of pilocarpine-loaded nanoparticles. J. Microencapsulation 20: 449-458 (2003).

PO004

Lipid nanoparticle systems containing curcumin: preparation and characterization

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INTRODUCTION

The oxidative damage and the inflammatory process are implicated in age-related neurodegenerative diseases including Alzheimer s Disease [1]. Amyloid plaques are known to cause oxidative damage and the generated free radicals may play a role in the pathophysiology of this disease. The yellow curry spice, curcumin, has both antioxidant and anti-inflammatory activities which confer significant protection against neurotoxic and genotoxic agents [1]. Moreover, curcumin inhibits the formation and extension of beta amyloid fibrils, and also destabilizes beta amyloid plaques that had already formed [2]. However, the use of this compound is limited by its low [3].

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are colloidal carriers potentially useful for poorly water soluble drugs for all the administration routes [4]. Intravenous administration of curcumin-loaded nanoparticles could potentially enhance drug blood circulation time and improve drug performance due to their ability to cross the Blood Brain Barrier (BBB) [4].

This paper describes the preparation of curcumin-loaded SLN and NLC, their characterization with regard to particle size distribution, zeta potential, loading capacity (LC%) and drug release profiles under several conditions.

EXPERIMENTAL METHODS

• Preparation of nanoparticles

Several curcumin-loaded SLN and NLC were prepared by using the precipitation technique. Briefly the selected lipid or mixture was heated to 5-10 °C above its melting point. Successively, curcumin (100 mg) and an ethanolic solution of Epikuron 200 were added to the melted lipid phase. The obtained hot solution was dispersed in twice-distilled water (2-3 °C) containing taurocholate sodium salt, by using an Ultra Turrax T25 at 6500 rpm for 10 minutes to obtain nanoparticles. Successively, nanoparticles were purified by dialysis, freeze-dried by a lyophilizer and characterized.

• Characterization of nanoparticles

The average diameter, polydispersity index (PDI) and zeta potential of nanoparticles were determined in twice-distilled water. An adequate HPLC method was developed to study the stability of drug in phosphate buffer (PBS) at pH 7.4/octanol and in human plasma, the curcumin LC% of nanoparticles and drug release profiles from loaded systems.

RESULTS AND DISCUSSION

In this work, several curcumin-loaded SLN and NLC were prepared. In particular, four different samples were prepared by using different lipid matrices: Imwitor (sample A), Imwitor-Miglyol (sample B), Precirol (sample C), and Precirol-Miglyol (sample D). In samples B and D, Miglyol was 20% w/w of the total lipid matrix. Table 1 shows the mean size, the PDI, the zeta potential and the LC% values of the obtained systems.

Table 1: Mean size, PDI, zeta potential and LC% values of samples

	Size (nm)	PDI	Zeta potential (mV)	LC% (w/w)
A	135.15	0.324	-24.1 ± 1.3	25.1
B	152.92	0.186	-16.9 ± 1.5	23.2
C	129.62	0.313	-21.5 ± 1.2	27.4
D	205.67	0.307	-26.6 ± 1.6	30.7

Data indicate that all the obtained systems possess nanometer diameters, with a good size homogeneity. Probably the higher size of batches B and D respect to A and C was due to oily nano-compartments within the solid lipid matrix. Zeta potential values were negative for all samples. Moreover, the high surface nanoparticle charge could reduce the occurrence of the aggregation phenomena and could assure a considerable stability of the obtained systems.

All batches had a great value of LC%, expressed as the percentage ratio between the weight of entrapped drug and the total weight of drug-loaded nanoparticles probably due to very lipophilic nature of curcumin. In vitro release kinetics in PBS at pH 7.4/octanol were very slow, in fact after 21 days the released drug didn't achieve 15% of the total drug entrapped (Fig. 1).

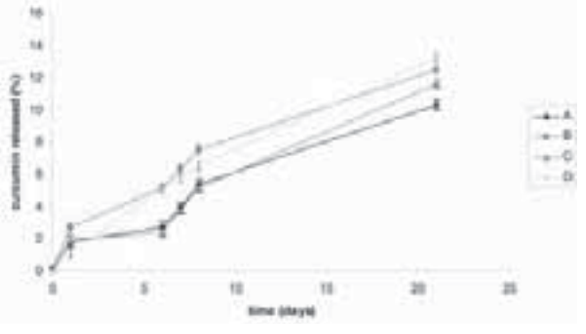

Figure 1: Release profiles of curcumin in PBS at pH 7.4/octanol at 37 ± 0.1 °C. Each value is the mean of three experiments.

Moreover, the drug amount that was not released within 21 days was found in nanoparticles (in this medium, curcumin was not degraded, data not shown).

Release studies were achieved, also, in human plasma as shown in Fig. 2.

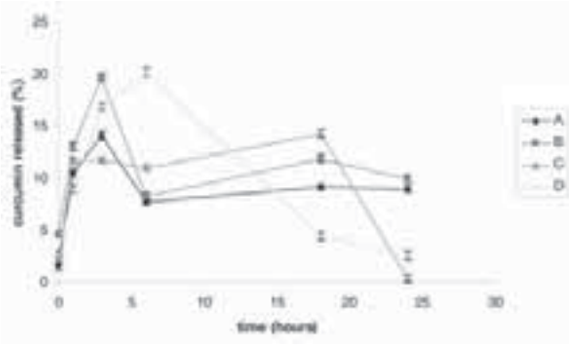

Figure 2: Release profiles of curcumin in human plasma at 37 ± 0.1 °C. Each value is the mean of three experiments.

The drug amount that was not released within 24 hours was found partially in nanoparticles (Fig. 3). The remaining amount not found, very probably, was degraded as reported by Wang et al. [5].

The data of release kinetics show that these systems are good carriers for curcumin and could be proposed for targeting to CNS. Biological studies are, actually, in progress to establish if curcumin loaded nanoparticles are active against oxidative stress and if the systems are biocompatible.

CONCLUSION

Curcumin loaded nanoparticles were prepared by precipitation technique. The obtained systems showed a size in the nanometer scale and had a negative surface charge. All batches had a great value of drug loading. The data of release kinetics showed that these systems are good carriers for curcumin and could be proposed for targeting to CNS.

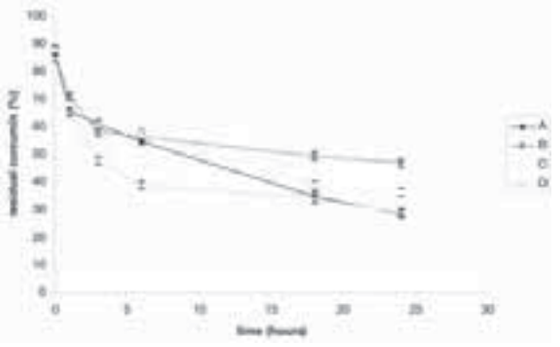

Figure 3: Residual curcumin amount found into nanoparticles after release. Each value is the mean of three experiments.

REFERENCES

1. Butterfield D.A. et al. Nutritional approaches to combat oxidative stress in Alzheimer's disease. The Journal of Nutritional Biochemistry 13: 444–461 (2002).
2. Ono K. et al. Curcumin as potent anti-amyloidogenic effects for Alzheimer's -amyloid fibrils in vitro. Journal of Neuroscience Research 75: 742-750 (2004).
3. Pak Y. et al. Sensitive and rapid isocratic liquid chromatography method for the quantitation of curcumin in plasma. Journal of Chromatography B 796: 339-346 (2003).
4. Béduneau A. et al. Active targeting of brain tumors using nanocarriers. Biomaterials 28: 4947–4967 (2007).
5. Wang Y.J. et al. Stability of curcumin in buffer solutions and characterization of its degradation products. Journal of Pharmaceutical and Biomedical Analysis 15: 1867-1876 (1997).

PO005

Cisplatin loaded SLN produced by coacervation technique

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INTRODUCTION

Solid lipid nanoparticles (SLN) are disperse systems suitable for drug delivery owing to their biocompatibility. We developed a new nanoparticles production technique, based on acidic coacervation of fatty acid salt micelles [1]. One of the main issues of this work is the further development of this technique, by studying other non ionic stabilizers than polyvynil alcohol (PVA), used in previous works: commercial polymers, like Pluronic® and new polymers, like modified dextrans (dexP), prepared basing on a literature synthesys [2], were tested. The molecular formula and substitution degree (τ) of these molecules are shown in figure 1.

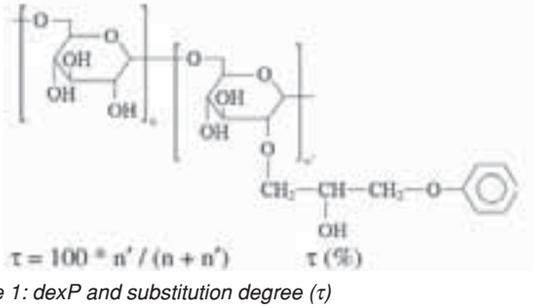

Figure 1: dexP and substitution degree (τ)

Cisplatin (cisPt) was chosen as model drug in order to obtain a sustained release and, consequently, a reduced tossicity by encapsulation within SLN [3].

CisPt, a hydrophilic molecule, was encapsulated within nanoparticles through hydrophobic ion pairing with sodium dioctylsolfosuccinate (AOT). As described in literature [4], this ion pair acts like a prodrug, since it is reversibile in normal saline, giving back original cisplatin.

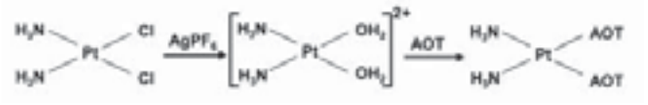

Figure 2: cisPt hydrophobic ion-pair

EXPERIMENTAL METHODS

Different amounts of stearic acid (SA) sodium salt, alone or in the presence cisPt-AOT, were dissolved at 47°C in an aqueous solution of a non ionic polymeric stabilizing agent. Then, an appropriate amount of a solution of lactic acid was added. After cooling SLN suspension was obtained. SLN were characterized by size distribution (LLS) and lipid melting point (mp) by DSC. Drug entrapment efficiency (EE) was determined on centrifuged SLN, washed with normal saline in order to remove adsorbed drug; cisPt was extracted from nanoparticles with normal saline after SLN dissolution in CH_2Cl_2 and determined by UV-HPLC (315 nm) after derivatization with diethyl-dithiocarbamate (DDTC) [5].

Drug release from SLN was performed on a suspension of nanoparticles in normal saline: at scheduled times the receiving phase was centrifuged and the supernatant analysed by HPLC.

RESULTS AND DISCUSSION

Prior to SLN drug loading, a preliminary study was performed to choose the acid to be used for coacervation: indeed its corresponding anion should not extract cisPt from a solution of cisPt-AOT in CH_2Cl_2, chosen as a model of the inner core of SA sodium salt micelles. As shown in table 1 lactate had the lowest extraction capacity and so lactic acid was chosen for the coacervation.

Table 1: cisPt extraction capacity of various anions

Anion	cisPt
Citrate	100%
Lactate	26%
Acetate	65
Phosphate	98%

1% SLN mean size and polydispersity, obtained using different stabilizers, are shown in table 2: increasing the dexP substitution degree, the SLN mean size decreases.

DSC analysis (figure 3) showed a peak nearly at 53°C for all the formulation, owing to different stabilizers.

Table 2: 1% SLN mean size and polydisperity

Formulation	Size (nm)	Poly.
1% SA SLN 1% PVA 9000	285,7	0,008
1% SA SLN 1% Pluronic® F68	275,2	0,015
1% SA SLN 1% Pluronic® F127	324,4	0,135
1% SA SLN 1% dexP(t=7%)	525,6	0,205
1% SA SLN 0.8% dexP(t=11%)	500,1	0,184
1% SA SLN 0.5% dexP(t=15%)	380,5	0,139
1% SA SLN 0.2% dexP(t=22%)	334,2	0,085

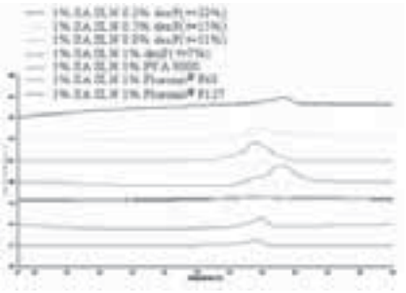

Figure 3 : 1% SLN DSC analysis

Drug EE was studied as a function of lipid matrix concentration by using PVA 9000 as stabilizer: an increase in EE was observed increasing lipid matrix concentration as shown in Figure 4.

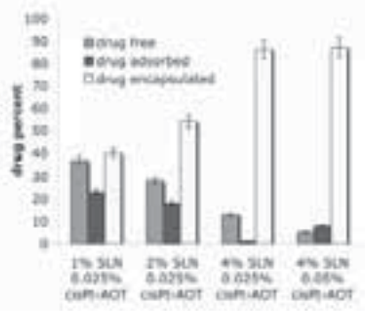

Figure 4 : drug EE of various % SA SLN

The effect of various stabilizers on drug EE by using 4% SA SLN is reported in figure 5: Pluronic® F68 causes a large decrease in EE compared to PVA 9000 and dexP(τ=22%).

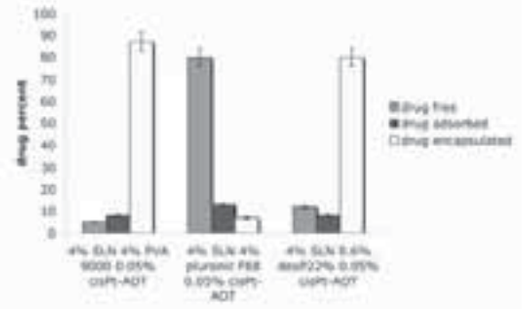

Figure 5 : drug EE of 4% SA SLN with various stabilizers

Drug release from nanoparticles was performed by using PVA 9000 and dexP(τ=22%) stabilized 4% SLN (0.05% cisPt-AOT): as shown in figure 6, the complete release of drug in normal saline was obtained within 24 hours.

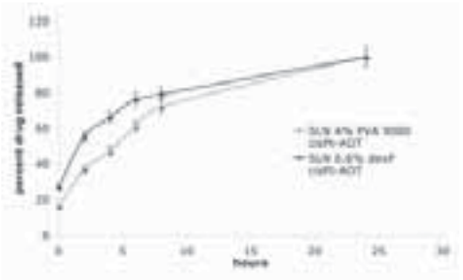

Figure 6: cisPt release from SLN in normal saline

CONCLUSIONS

Coacervation method is a versatile method to produce cisPt loaded SLN of stearic acid through hydrophobic ion pairing. CisPt, chosen as hydrophilic model drug, was effectively encapsulated in nanoparticles, which act like a sustained release system. Further studies are necessary to verify citotoxicity and in vivo behaviour of the SLN loaded drug.

References

1. L. Battaglia, M. Trotta, R. Cavalli Italian patent n° TO2007A000411
2. C. Rouzes, R. Gref, M. Leonard, A. De Sousa Delgado, E. Dellacherie. Surface modification of poly(lactic acid) nanospheres using hydrophobically modified dextrans as stabilizers in an o/w emulsion/evaporation technique. Biomedical Material Research 50: 557–565 (2000)
3. K. Avgoustakis, A. Beletsi, Z. Panagi, P. Klepetsanis, A.G. Karydas, D.S. Ithakissios. PLGA–mPEG nanoparticles of cisplatin: in vitro nanoparticle degradation, in vitro drug release and in vivo drug residence in blood properties. Journal of Controlled Release 79: 123–135 (2002)
4. Lixin Feng, Alexandra De Dille, Vicky J. Jameson Leia Smith, William S. Dernell, Mark C. Manning. Improved potency of cisplatin by hydrophobic ion pairing. Cancer Chemotherapy and Pharmacology 54: 441–448 (2004).
5. Rajagopalan Raghavan, Mark Burchett, David Loffredo, and Jo Anne Mulligan. Low-Level (PPB) Determination of cisplatin in cleaning validation (Rinse Water) samples. II. A. High-Performans Liquid Chromatographic Method. Drug Development and Industrial Pharmacy 26 (4): 429-440 (2000).

PO006

Preparation and characterization of stearic acid loaded nanostructred lipid carriers

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ABSTRACT SUMMARY

Stearic acid loaded solid lipid nanoparticles (SLN) of repaglinide were prepared by modified solvent injection techniques with using poloxamer F-68 as surfactant. The particle size, zeta potential, encapsulation efficiency, morphological character by scanning electron microscopy and in-vitro release characteristics of drug loaded SLNs were characterized.

INTRODUCTION

Amongst the various routes of drug delivery, oral route is perhaps the most preferred to the patient and the clinician alike but approximately 40% of drug candidates have poor water solubility and the oral delivery of such drugs was frequently associated with implications of low bioavailability, hepatic first pass metabolism, enzymatic degradation, high intra- and inter subject variability, and lack of dose proportionality. Nowadays, the interest of solid lipid nanoparticles (SLN) to improve the oral bioavailability of poorly water soluble drugs were well known and documented with various drugs: Clozapine, cyclosporine, nifedipine, vinpocetine, and halofantrine. SLN are enhanced lymphatic transport of the drugs reduces the hepatic first metabolism and improve bioavailability, because intestinal lymph vessels drain directly into the thoracic duct, further in to the venous blood, thus by passing the portal circulation (Porter and Charman, 2001). Repaglinide (RG), a carbamoylmethyl benzoic acid derivative belong to a new class of oral hypoglycaemic agents known as the meglitinide analogues. The mean oral bioavailability of repaglinide is around 50 %. RG is highly bound to plasma proteins (>98%). Repaglinide is rapidly cleared from the blood-stream with a terminal elimination half-life ($t_{1/2}$) of <1 hour (Davis et al., 2001).Therefore, the aim of the proposed research work will be to prepare and characterize repaglinide solid lipid nanoparticles with controlled release intended for peroral administration, and also to improve the bioavailability after oral administration.

EXPERIMENTAL METHODS

SLNs were prepared by modified solvent injection technique (Schubert et al.,2003).RG and stearic acid (SA) were dissolved in acetone under continuous stirring by using rotamantle at room temperature .An aqueous phase was prepared by dissolving Poloxamer 407 (PL 407) in distilled water, and this was heated to the same temperature as the organic phase. The resulting organic solution was rapidly injected through an injection needle into stirred aqueous phase (1000 rpm).The resulting suspension was stirred at 75^{0}C under continuous stirring (1000 rpm) for 4 hrs by using an rotamantle.Then the organic solvent was removed from the dispersion and a translucent nanoemulsion was obtained. The formulation strategies:

Table 1: Formulation strategies

Sr. No.	Batch code	Drug (mg)	SA (mg)	PL 407 (mg)
1	MR_1	40	400	200
2	MR_2	40	200	200
3	MR_3	40	200	100

The SLNs were characterized by particle size analyzer, zeta potential, entrapment efficiency (EE), surface morphology by scanning electron microscope (SEM) and in-vitro drug release (%DR) study. The EE and %DR were analyzed by spectrophotometer at 282 nm.

RESULTS AND DISCUSSION

Table 2 shows the particle size, PI and zeta potential of SLNs batches prepared by solvent injection method. No significant difference in particle size was observed between MR_2 and MR_2 but MR_1 has more particle size than two batches.This can be due to the use of higher concentration of stearic acid as increase the lipid content in formulation the particle size is increase. The zeta potential and stability of nanoparticle systems are closely related. Measurement of zeta potential can predict the stability of nanoparticles systems. The zeta potential of the three batches in this study is shown in table 10. MR_1 and MR_2 are more stable than MR_3. MR_1 has highest EE which showed that decrease in the concentration of the lipid led to the decrease in the drug EE of the SLNs.This can be attributed to the higher solubility of drug in the higher lipid concentration.

Table 2: shows the particle size, polydispersity index (PI), zeta potential and entrapment efficiency (EE) of all prepared batches

Batch code	Particle size (nm)	PI	Zeta potential (mV)	EE (%)
MR_1	748 (3.3)	0.560 (0.009)	-20.45 (1.23)	50.26
MR_2	501 (2.5)	0.430 (0.050)	-18.03 (2.80)	45.01
MR_3	478 (5.3)	0.398 (0.020)	-15.9 (0.89)	44.78

The MR_3 showed the controlled release of SZ for almost 7 hrs and it was significantly better than MR2 and MR_3. **MR_3** showed the burst release might be because of higher particle size (Fig. 1).

The Fig. 2 showed the amorphous nature of repaglinide loaded SLNs.

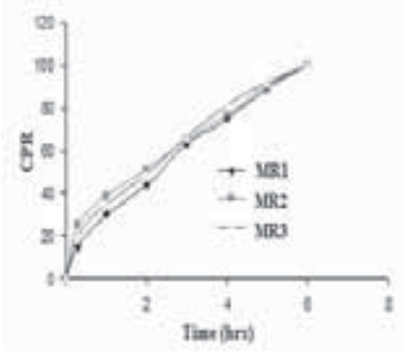

Figure 1: In vitro release

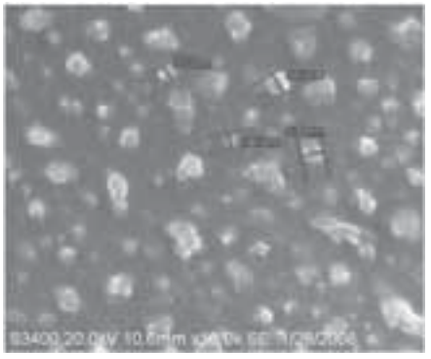

Figure 2: SEM of repaglinide loaded SLNs

CONCLUSION

The method resulted in consistent production of medium size nanoparticles in the range of 450-750 nm with narrow size distribution and good entrapment efficiency. The zeta potential is around -15 to -20 mV which is not good for nanoparticles stability. For stable nanoparticles the zeta potential should be more than – 30 mV or near to this. The future objectives of this work to enhance the stability of formulation and do the pharmacodynamic and pharmacokinetics study.

ACKNOWLEDGEMENT

We thankful for UGC for providing Senior Research Fellowship.

References

1. C.J.H. Porter and S.A. Charman, Lymphatic transport of proteins after subcutaneous administration. *J. Pharm. Sci.* 89 (2000), pp. 297–310.
2. E.J. Mayer-Davis, S. Levin, R.N. Bergman, R.B. D Agostino, A.J. Karter and M.F. Saad, Insulin secretion, obesity, and potential behavioral influences: results from the Insulin Resistance Atherosclerosis Study (IRAS). *Diabetes/Metabolism Research and Review* 17 (2001), pp. 137–145.
3. M. A. Schubert , C. C. Müller-Goymann, Solvent injection as a new approach for manufacturing lipid nanoparticles – evaluation of the method and process parameters. *Eur. J. Pharm. Biopharm.* 55(2005), pp. 125–131.

PO007

Ascorbyl palmitate nanosuspensions: factors affecting the physical stability

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INTRODUCTION

Ascorbyl palmitate is a lipophilic antioxidant substance used to increase the shelf life of nutritional and cosmetic products. Moreover, when incorporated into the cell membranes of human red blood cells, ascorbyl palmitate has been found to protect them from oxidative damage and to protect alpha-tocopherol (a fat-soluble antioxidant) from oxidation by free radicals [1]. Its solubiluty and activity is mainly dependent on the particle size and surface area from which the substance can be dissolved.

In nanonization process the drug powder is transferred to drug nanocrystals with typical sizes around 200-600 nm. Drug nanocrystals are composed of 100% drug, there is no carrier material as in polymeryc nanoparticles. Dispersion of drug nanocrystals in liquid media leads to a nanosuspension. In general, the dispersed particles can be stabilized, e.g. by surfactants or polymeric stabilizers. Dispersion media can be water, aqueous solutions or non-aqueous media (e.g. liquid polyethylene glycol (PEG), oils). Different technologies are used for nanonisation of drugs, howeever drug nanoparticles are most commonly produced by milling or by high-pressure homogenization using preferentially piston-gap homogenizers [2,3,4].

The aim of this study was to prepare stable nanosuspensions with the antioxidant substance ascorbyl palmitate for cosmetic formulations and to evaluate their physical stability.

MATERIALS AND METHODS

• Materials

Acsorbyl palmitate was purchased from Denk Feinchemie GmbH, Germany. Polysorbate 80 (Tween 80 V Pharma, Uniqema, Belgium) was used as a nanosuspension stabilizer and phenoxyethanol/ethylhexylglyceryn (Euxyl PE 9010, Schülke & Mayr, Germany) was used as nanosuspension preservative.

• Methods

Nanosuspensions with 12 or 6 % ascorbyl palmitate, containing 1, 1.5, 2 or 3% Tween 80 (stabilizer) and 1 % Euxyl PE 9010 (preservative) were prepared using an agitated ball mill Bühler PML-2 (Bühler AG, Switzerland) with 0.4-0.6 mm yttria stabilized zirconium oxide beads. Homogenization was performed using a lab scale high pressure homogenizer Lab 40 (APV Deutschland GmbH, Germany), at different homogenization pressures: 300, 500 and 1500 bar. Particle size analysis was performed by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, UK) and laser diffractometry using a Mastersizer 2000 (Malvern Instruments, UK). For stability evaluation samples were stored in a refrigerator (4-8°C), at room temperature and 40°C and analyzed after 7 days, 1 month, and 3 months.

DSC analyses of ascorbyl palmitate powder and nanosuspension were performed using a differential scanning calorimeter DSC 821^e (Metler Toledo, Switzerland). Samples containing about 1-2 mg of ascorbyl palmitate were weighed and sealed in high pressure stainless steel pans and analysed at heating and cooling rate of 5deg/min in a dynamic nitrogen atmosphere. An empty pan was used as a reference.

Optical analysis of nanosuspensions was performed using a light microscope Leitz Orthoplan, Leitz Germany)

RESULTS AND DISCUSSION

Ascorbyl palmitate crystallizes in the form of needles (Figure 1) with a melting range of 107-117°C. Particle size of ascorbyl palmitate decreased as a function of the number of ball milling cycles. A significant particle size reduction was obtained already after first ball milling cycle. The mean particle size further decreased with more cycles employed (Figure 2) and a PCS particle diameter of about 400 nm and laser diffractometry diameter 50% of 0.117 μm and diameter 90% of 0.200 μm were finally reached.

The ball milled nanosuspension was further homogenized using high pressure homogenization. Increasing the homogenization pressure yielded nanosuspension with smaller particles size: 350, 300 and 250 nm after high pressure homogenization at pressures 300, 500 and 1500 bar, respectively (Figure 3). At higher homogenization pressures and higher concentration of stabilizer Tween 80 the particles size increased slightly but impact on the particle size of the nanosuspensions was not significant.

DSC results confirmed the crystalline ascorbyl palmitate in the nanosuspensions. Melting peaks of ascorbyl palmitate were detected in DSC scans of ball milled and high-pressure homogenized nanosuspensions, however the melting temperature and melting enthalpy of ascorbyl palmitate in nanosuspension was decreased due to the presence of stabilizer Tween 80 and preservative Euxyl PE 9010.

The nanosuspension obtained by this process combination showed a limited tendency of increasing the particle size after prolonged storage time at 4-8°C.

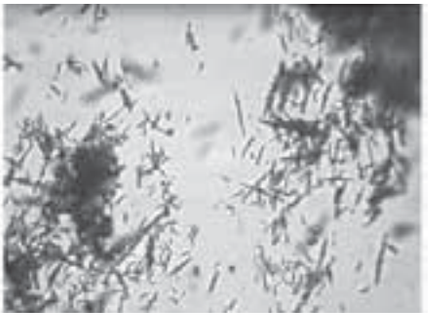

Figure 1: Ascorbyl palmitate powder.

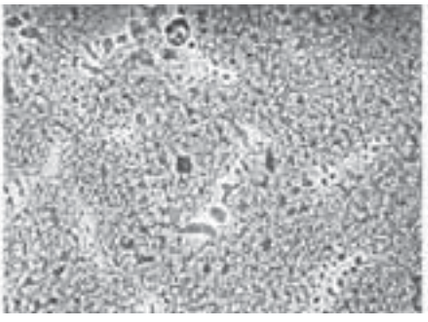

Figure 2: Ascorbyl palmitate nanosuspension after 5 cycles of ball milling

It was also found out, that the stability of nanosuspension regarding particle size is increased with lower concentration of ascorbyl palmitate in the nanosuspension.

The high-pressure homogenized nanosuspensions were more stable and the particle size increase after prolonged storage time was smaller compared to ball milled nanosuspensions. In further studies nanosuspensions stored at 4-8°C were found more stable than those stored at room temperature or 40°C.

Figure 3: Ascorbyl palmitate nanosuspension after ball milling and high pressure homogenization at 500bar

High-pressure homogenization was found the major factor affecting the physical stability of nanosuspensions. Furthermore, higher homogenization pressure and consequently decreased particle size of nanosuspension provide more stable nanosuspension.

CONCLUSIONS

A combination of ball milling and high pressure homogenization was proved to be very efficient technology for preparation of ascorbyl palmitate nanosuspensions. Nanosuspension concentration and homogenization pressure were found major factors affecting the physical stability of nanosuspensions.

References

1. Ross, D. et al. Ascorbate 6-palmitate protects human erythrocytes from oxidative damage. Free Radical Biology and Medicine. 26: 81-89 (1999).
2. Müller, R. H., Junghanns, J.-U., Drug nanocrystals/nanosuspensions for the delivery of poorly soluble drugs, in: Nanoparticulates as Drug Carriers (V. P. Torchilin, ed.), 307-328 (2006).
3. Müller, R. H., Akkar, A., Drug nanocrystals of poorly soluble drugs, in: Encyclopedia of Nanoscience and Nanotechnology (H. S. Nalwa, ed.), American Scientific Publishers, 627-638 (2004).
4. Keck, C. M., Müller, R. H., Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation, Eur. J. Pharm. Biopharm. 62: 3-16 (2006).

PO008

Tablets for subsequent filling with nanoparticle suspensions using ultrasound

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INTRODUCTION

For achievement of a therapeutical effect, active pharmaceutical ingredient (API) has to be incorporated into appropriate therapeutical system. Tablets are preparations that consist of single dose of one or more active ingredients. From the patient point of view for both therapeutic and prophylactic response, peroral aplication is most desirable manner of drug application. It is desirable for patients also since it avoids the disadvantageus parenteral injections. In general, conventional tablets are prepared by mixing of powders or granulation of excipients and API before compression. Advantage of direct tabletting in comparison to granulation is in lower production costs. For the tablet production less time, equipment and space, less validations and energy is needed. Also disadvantages of direct tabletting exist:

- it is difficult to ensure equal distribution of components and prevent demixing of formulations with low content of active pharmaceutical ingredients,

- fillers used for direct tabletting are more expensive than fillers used for granulation,
- limitations of production of colorized tablets,
- dusting problems.

A novel method for tablet production employs addition of API solution into empty tablets that consist only of exipients. Porous tablets are made by compression of excipients without API which is subsequently added as a melt, solution in an apropriate solvent or susupension [1,2].

Nanoparticle production is one of the possibilities to increase bioavailability of the API after peroral aplications. They can be administered as suspension or in a solid dosage form (e.g. tablet). Prior tabletting nanoparticles have to be appropriatelly dried using spray-drying or frezing-drying. In this investigation alternative way of drying by filling placebo tablets with a model nanoparticles suspension using ultrasound was investigated.

EXPERIMENTAL METHODS

• Tablet preparation

Silicified microcrystalline cellulose (Prosolv HD90, JRS Pharma, Rosenberg, Germany) was used for compression of placebo tablets with a porosity around 40%, mass 400 mg, diameter 12 mm and hardness 85 N in a SP3000 (Kilian, Köln, Germany) tablet press.

• Filling experiments

Model ZnS, $BaSO_4$ or TiO_2 nanoparticles with average size of 170, 190 and 600 nm respectivelly were dispersed in acetone (20 % concentration). Placebo tablet was immersed into suspension for 10 s, 30 s or one minute. Longer immersion time than one minute caused dissintegration of the tablet. Alternativelly tablets were filled by immersion into a 20% nanoparticles suspension in aceton using a magnetic stirrer. Filled tablets were dried at 60 °C in a vacuum chamber.

• Particle size measurement

The mean diameter of the model nanopatricles was measured with laser diffraction system Mastersizer S (Worcestershire, UK) employing lense 300RF (0.05-900 μm).

• EDS analysis

Energy dispersive x-ray analysis was used for elemental composition of a crossection of the tablets filled with nanoparticles in SEM microscope Supra 35VP (Carl Zeiss, Oberkochen Germany) and EDS inca 400 (Oxford instruments, Oxfordshire, UK). The prepared sample was carbon coated prior to analysis to provide a conductive film.

RESULTS AND DISCUSSION

Mass of nanoparticles in the tablets using magnetic stirrer ranges from 2.8 mg for $BaSO_4$ to 5.2 mg for TiO_2. Results are relativelly low since particles adsorbed only at the surface and large opened pores of the tablet. When ultrasound was applied vibrations enabled particles to reach much deeper into the tablet increasing the filling yield (Table 1). In this case maximal mass obtained after drying was from 9.3 mg for TiO_2 to 15.3 mg for $BaSO_4$. These results are in correlation with particle size. $BaSO_4$ and ZnS having small particle size reached much smaller pores in the tablet than TiO_2 (Table 1). Amount of nanoparticles incorporated in the tablets increased also with the sonication time (Fiure 1). Amount of nanoparticles incorporated was increased also after second filling what indicates multilayered deposition. How deep particles reached into the matrix, was investigated using EDS analysis. Tablets were cut perpendicular to the thickness and analysed from the edge to the middle. It was found out that when magnetic stirrer was used for filling particles penetrated only about 50 μm into the tablet. When ultrasound was used this distance was increased up to 400 μm confirming that tablet structure, having large and thin capillaries, disables penetration of particles into whole matrix.

Table 1: Particle size and amount of nanoparticles in the tablets filled using magnetic strirrer and ultrasound for one minute in acetone suspension (20%).

Nanopart.	Particle size	Magnetic stirrer	Ultrasound
$BaSO_4$	190 nm	2.8 ± 0.8 mg	15.3 ± 2.5mg
ZnS	170 nm	3.6 ± 1.4 mg	15.1 ± 0,3mg
TiO_2	600 nm	5.2 ± 0,4 mg	9.3 ± 0.7 mg

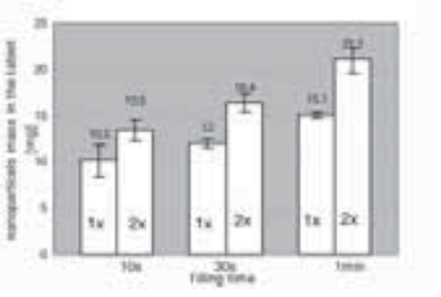

Figure 1: Mass of incorporated ZnS nanoparticles after first (1x) and second filling (2x) using different sonnication time.

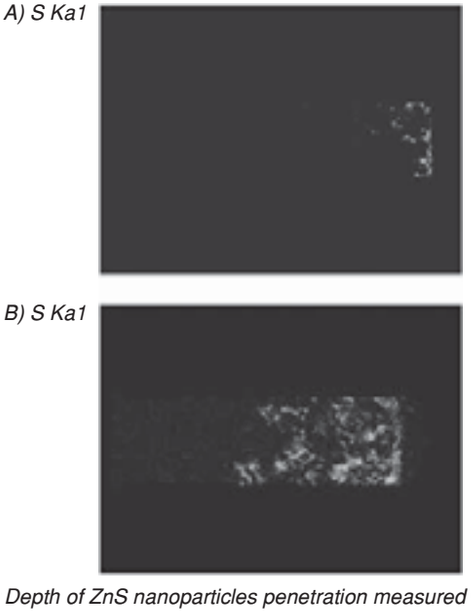

Figure 2: Depth of ZnS nanoparticles penetration measured with EDS analysis for tablets filled using megnetic stirrer (A) and ultrasound for one minute (B).

CONCLUSIONS

It was shown that filling of placebo tablet with nanoparticles suspension can be used as drying method for nanoparticles with a low dose active pharmaceutical ingredients or with antigens for oral immunization and vaccination against systemic infections. These new tablets have a low cost, are easy to administer, increase patient confort and can increase patient compliance and could be suitable also for use in developing countries.

References

1. Planinšek O. Porozne tablete za naknadno polnjenje z zdravilno učinkovino, P-200600081.
2. Holm P. et al. Porous tablets as carriers for liquid formulations. WO 2006/000229.

PO009

Novel nanocomposite silica based materials for stabilization and controlled release of drug

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INTRODUCTION

A diversity of pharmaceutical carrier systems for controlling temporal or distributional (site-specific) drug delivery have been developed during the past decades. These offer numerous advantages when compared to conventionally administered drugs in dosage forms, such as improved efficiency and reduced toxicity (1).

A promising technique for preparation of controlled delivery systems is the sol-gel procedure, which involves the manufacture of an inorganic matrix through the gelation of colloidal suspension (sol) in mild conditions. This technique also allows incorporation of highly sensitive molecules, like proteins and peptides into the gel (3,4).

There are many approaches to the sol-gel synthesis of hybrid materials, most of which involve a combination of two or more precursor species. The addition of organic modifiers is widely used for modifying the morphology and surface characteristics of silica xerogels prepared with the precursor tetraethoxysilane (TEOS), which is the most often used silica precursor.

The aim of our work was to show that incorporation of a model drug into a porous solid matrix with pores in the range of few nm should lead to composites in which the drug would be in the amorphous rather than in the crystalline state. The amorphous form of drug has been interesting due to its specific characteristics such as a faster dissolution and higher reactivity when compared to the crystalline form. Of course, when the amorphous drug is encapsulated into a solid matrix, these characteristics may be changed. We show that the dissolution rate is significantly decreased if nifedipine is incorporated into a uniform silica matrix. By appropriate modification of the composition of silica precursors, we can again increase the average rate of dissolution nifedipine from the silica-drug composites. Finally, a mechanism leading to this increase is proposed and discussed.

EXPERIMENTAL

As a microporous solid matrix silica was selected, while nifedipine served as a model drug. The silica–drug composites were prepared using a sol–gel procedure at conditions which yielded pores in the range 2–3 nm. To tune the properties of composites, two silica precursors were combined: tetraethoxysilane (TEOS) and bis-1,2-(triethoxysilyl)ethane (BTSE).

The prepared carrier systems were analysed using N_2-adsorption, X-ray powder diffaction, thermal analysis, scanning electron microscopy, laser diffraction, fourier transform infrared spectroscopy and dissolution testing.

RESULTS AND DISCUSSION

In all composites the amorphous state of nifedipine was proven using analytical and theoretical methods. The values of critical dimensions as calculated using the proposed model, which is published elsewhere, are displayed in Table I (5).

Table 1: The comparison of the calculated and the experimental gained radii of the crytical nuclei, where $r_{experimental}$ represent the average pore radius of the prepared samples, $r_{am.phase}$ the radii of the crystalline crytical nuclei formed in the amorphous phase and $r_{supersat}$. represents the radii of the crystalline crytical nuclei formed in a supersaturated solution (where S represents the degree of supersaturation)

	$r_{experimental}$ [nm]	$r_{am.phase}$ [nm]	$r_{supersat}$ [nm]	
			S=1,5	S=4,5
Spherical nuclei	1,20	2,9	2,6	9,7
Cubical nuclei	1,44	4,5	6,8	24,4

It can be seen that all calculated values are significantly higher than the average dimensions of pores determined experimentally for the present silica matrices. In other words, the radii of pores in the present samples are too small to allow crystallization either from saturated solution or

from the amorphous solid phase that has already formed within the pores.

Drug incorporation into purely TEOS-based silica decreased significantly the release rate. Addition of BTSE made the structure more brittle, which led to increased drug dissolution rate, while the amorphicity was preserved. The dissolution behaviour was succesfully explained using a combination of the Noyes–Whitney and power law model.

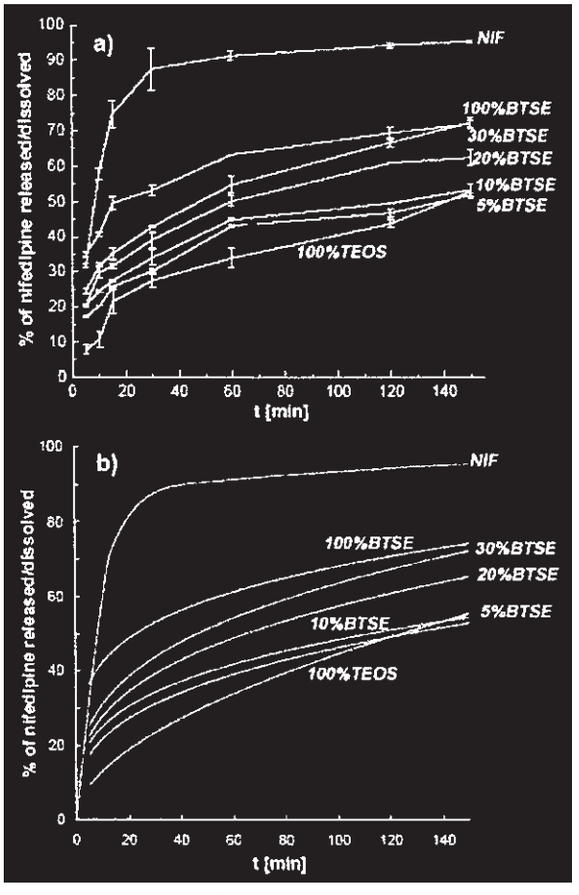

Figure 1: Percentage of NIF released as a function of time for the modified and nonmodifed samples. b) Model curves obtained by a quantitative analysis of the measured dissolution curves (combination of the Noyes-Whitney and the power law).

CONCLUSIONS

The occurrence and stability of amorphous NIF are explained by the fact that NIF is spatially constrained within pores of an average size of about 2.4 nm while for crystallization a space larger than ca. 3.4 nm (in diameter, if spherical) is theoretically predicted. The structural and mechanical properties of the prepared xerogels were controlled by varying the molar ratio between two silicate precursors: tetraethoxysilane (TEOS) and (bis-1,2-(triethoxysilyl)ethane, BTSE). With increasing BTSE content the samples became more brittle and degraded faster when immersed into a solution. Thus, about 30% of the sample containing only BTSE degraded within first 5 minutes of immersion.

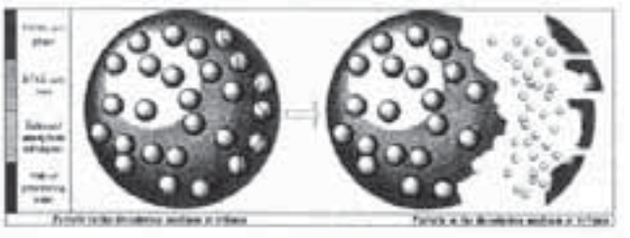

Figure 2: Schematics of the proposed release scenario. Before immersion into solvent, NIF (small yellow circles) is packed inside the matrix. The solvent breaks partly the xerogel matrix releasing a certain amount of NIF initially captured inside the matrix. This part dissolves rapidly while NIF that remains encapsulated is released slowly.

The observed release patterns could be interesting for therapies requiring a high initial drug concentration in blood plasma, which is maintaned by a following slower release rate of the remaining drug.

References

1. A. Rösler, G. W. M. Vandermuelen. Adv. Drug Delivery Rev. 53: 95 (2001).
2. R. Jain, N.V. Shah. Controlled drug delivery by biodegradable polyester devices: different preparative approaches. Drug. Dev. Ind. Pharm. 24: 703-727 (1998).
3. S.B. Nicoll, S. Radin. In vitro release kinetics of biologically active transforming growth factor-1 from novel porous glass carrier. Biomaterials 18: 853-859 (1997).
4. E. M. Santos, S. Radin. Sol-gel derived carrier for controlled release of proteins. Biomaterials 20: 1695-1700 (1999).
5. A. Godec., U. Maver, M. Bele, O. Planinšek, S. Srčič, M. Gaberščček, Janez Jamnik. Vitrification from solution in restricted space: Formation and stabilization of amorphous nifedipine in a nanoporous silica xerogel carrier. Int. J. Pharm. 343: 131-140 (2007)

PO010

Tablets compressed from spray dried celecoxib nanosuspension

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INTRODUCTION

A significant proportion of drugs on the market are poorly soluble and it is expected that this will be even more pronounced in the future (1). The research on colloidal drug delivery systems may be the hottest field in pharmaceutics in the last several decades. Among these colloidal systems, drug nanosuspensions have been paid more and more attention as a promising approach due to its pharmaceutical advantages and pharmacoeconomics value (2). Nanosuspension engineering processes currently used are precipitation, pearl milling and high pressure homogenization, either in water or in mixtures of water and water-miscible liquids or non-aqueous media (3, 4).

High pressure homogenization is mechanical process used to prepare suspensions of nanometer-sized particles of poorly water soluble drugs. The formation of nanosuspension is based on the cavitation forces created in high pressure homogenizers such as the piston gap homogenizer. In the process, the poorly water soluble drug is first dispersed in an aqueous surfactant solution by high speed stirring, and the suspension is than passed through a high pressure homogenizer (3, 4).

Celecoxib is a selective COX-2 inhibitor which is mostly used in treatment of osteoarthritis, rheumatoid arthritis and acute pain. It is classified as a class II substance according to biopharmaceutical classification system. Celecoxib has low aqueous solubility which contributes to high variability in absorption after oral administration. Thus, it is important to enhance the solubility and dissolution rate of celecoxib to improve its overall oral bioavailability (5, 6, 7).

The nanosuspension was prepared by emulsion diffusion technique. PVP K-30 and SDS were used as stabilizing agents. After the preparation the lactose was added and the nanosuspension was spray dried and further compressed to tablets. The nanosuspension was evaluated by laser diffraction. Scanning electron microscopy was used to characterize the surface of compressed tablets.

EXPERIMENTAL METHODS

Preparation of celecobib nanosuspension

Nanosuspension was produced by emulsion/diffusion method using ethylacetate. 800 mg of celecoxib was dissolved in 10 ml of ethyl acetate, poured into 50 ml 1,6 % w/w water solution of SDS/PVP K-30 (1:1) and stirred with Ultra Turrax T25 (Janke & Kunkel, IKA Labortechnik, Germany) for 2 minutes at 20.000 rpm. The emulsion was further homogenized by high pressure homogenizator (APV 2000, Invensys, Denmark) at 700/70 bars for 5 minutes, diluted with 250 ml of water and continued at the same pressure for 10 minutes to diffuse the organic solvent and convert the droplets into solid particles.

Particle size

Particle size of nanosuspension was analyzed by laser diffraction (LD) using Mastersizer S apparatus equipped with a small sample dispersion unit (Malvern Insruments, Worcestershire, UK). LD yields the volume size distribution and can measure particles in the size range 0,1 to 2000 μm.

Spray drying

Nanosuspension with dissolved lactose was converted to a dry state using spray drier Büchi B290 (Switzerland). The final dry product consisted of lactose, celecoxib, PVP and SDS in ratio 4:2:1:1. The inlet T of air was 160 °C. The aspirator and pump were set to 100 % and 15 % respectively.

Tableting process

Killian SP 300 (Köln, Germany) was used to compress tablets of spray dried nanosuspension. As a reference physical mixture of all components in the same ratio (PM) and spray dried excipients with raw celecoxib (SDEC) were compressed to form 400 mg tablets with 100 mg of celecoxib. To obtain tablets with hardness 50 to 60 N appropriate compaction forces were applied. Prior to tableting 2 % Mg-stearat was added to a tableting mass.

Scanning electron microscopy

Surface morphology of compressed tablets was studied by scanning electron microscopy (SEM, SUPRA 35 VP, Carl Zeiss).

RESULTS AND DISCUSSION

Characterization of nanosuspension

Particles in celecoxib nanosuspension were much smaller compared to raw celecoxib determined by laser diffraction (Table I.). The particle size was also determined after spray drying process and only D (v, 0.9) changed, probably because of aggregates and impurities from the process.

Table 1: Particle size of nanosuspension and raw celecoxib determined by laser diffraction.

Size (μm)	D (v, 0.1)	D (v, 0.5)	D (v, 0.9)
Raw celecoxib	1,70	4,10	11,24
Nanosuspension **before** spray drying	0,14	0,36	1,29
Nanosuspension **after** spray drying	0,13	0,36	1,84

Compaction and characterization of NS tablets

Dry nanosuspension (NS) was compacted to tablets with very low forces. Higher forces were needed to compact spray dried excipients with celecoxib (SDEC) and the highest forces were applied for physical mixture (PM) where hardness 55 N was reached at compaction force 16,9 kN (data not shown).

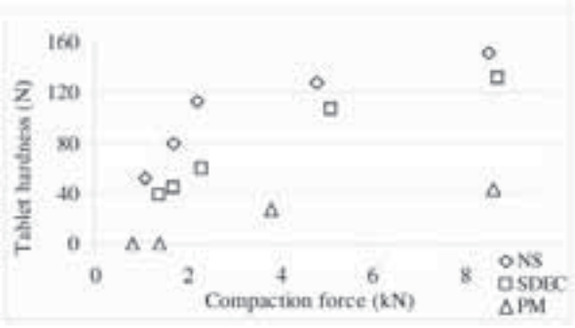

Figure 1: Correlation between compaction forces and tablet hardness when compacting tablets from spray dried nanosuspension (-◇-NS), spray dried excipients with raw celecoxib (-□-SDEC) and physical mixture of excipients and celecoxib (-△-PM).

SEM images showed high porosity and very small particles on the surface of the NS tablet (Figure 2b). Smaller celecoxib particles were also included in the spray dried mixture of lactose, PVP and SDS. The surface of physical mixture tablet was smoother due to high compaction forces (Figure 2a).

CONSLUSIONS

High pressure homogenization method was successfully used to prepare a celecoxib nanosuspension with PVP K-30 and SDS. It was spray dried and compacted to tablets with desired hardness at very low com

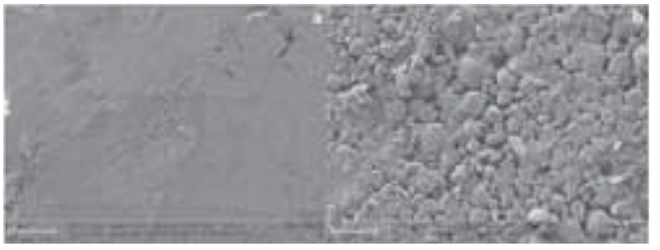

Figure 2: SEM images of physical mixture (a) and dry nanosuspension (b) tablet surface. Size bars represent 5 μm.

paction forces. The nanosuspension tablets possess higher porosity which leads to faster disintegration and higher dissolution rate (data not shown). Another benefit of low compaction forces is also lower apparatus loading.

References

1. Sigfridsson, K., Forssén, S., Holländer. P., de Verdier, J. 2007. Eur. J. Pharm. Biopharm. 67, 540-547.
2. Gao, L., Zhang, D., Chen, M., et al., 2008. Int. J. Pharm. 325, 321-327.
3. Hu, J., Johnston, K.P., Williams III, R.O., 2004. Drug. Dev. Ind. Pharm. Vol 30, No. 3, 233-245.
4. Kocbek, P., Baumgartner S., Kristl, J., 2006 Int. J. Pharm. 312, 179-186.
5. Chawla, G., Gupta, P., Thilagavathi, R., 2003. Eur. J. Pharm. Sci. 20 305-317.
6. Lu, G.W., Hawley, M., Smith, M. et al., 2005. Pharm. Sci. , 305-317.
7. Paulson, S.K., Vaughn, M.B., Jessen, S.M. et al., 2001. J. Pharm. Exp. Ther. Vol. 297, Issue 2, 638-645.

PO011

High celecoxib-loaded nanoparticles prepared by vibrating nozzle device

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INTRODUCTION

Nanoparticles (NP) show great potential as a means of delivering a drug to its site of action efficiently, thereby improving efficacy and minimizing unwanted toxic effects. In general, hydrophobic drugs could be incorporated into NP using emulsification-diffusion (1), solvent-evaporation (2), salting-out method (3) or nanoprecipitation method (4). In comparison with other techniques nanoprecipitation does not require toxic solvents and a source of external energy but it is often inferior in terms of achievable drug loading.

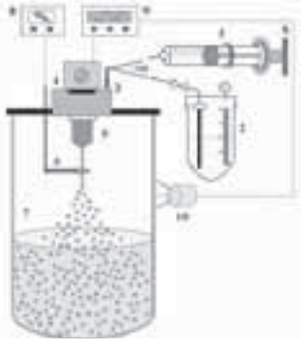
The vibrating nozzle device provides the opportunity for controlled encapsulation of drugs, animal cells, plant cells, microorganisms and enzymes mainly in alginate beads. The technology is based on the principle that a laminar liquid jet is broken into equaly sized droplets by a superimposed vibration. When passing through an electrical field between the nozzle and the electrode the surface of the droplets gets charged. Due to electrostatic repulsion forces the one-dimensional droplet chain is transformed in a funnel-like multiline stream. This prevents droplets from hitting each other in flight and when they enter the hardening solution.

The aim of this work was to prepare NP with high drug loading and entrapment efficiency by vibrating nozzle device. The influence of different polymers, initial drug/polymer ratios and stabiliser concentration on NP mean diameter, encapsulation efficiency, drug loading and in vitro celecoxib release was investigated in order to achieve the highest drug loading and entrapment efficiency.

EXPERIMENTAL METHODS

• Preparation and characterization of NP

PLA/PLGA (Resomer 202H, RG 502 and 752) and celecoxib were dissolved in acetone and dispersion was pumped through a 500 m nozzle of encapsulator and allowed to drop into aqueous polyvinyl alcohol (PVA) solution which was stirred magnetically. The experiments were performed at flow rate 9 ml/min, vibration frequency 482 Hz, amplitude 3.5 and the voltage 930 V. After preparation the organic solvent was evaporated at room temperature and NP were freeze dried. The mean particle size was estimated by a photon correlation spectroscopy and zeta potential by laser Doppler anemometry using a Zetasizer 3000 (Malvern, UK). The amount of celecoxib encapsulated per unit weight of NP was determined by direct determination of celecoxib in the prepared NP by HPLC analysis.



Legend:
1 Syringe with organic phase
2 Delivery bottle with organic phase
3 Pulsation chamber
4 Vibration system
5 Nozzle
6 Electrode
7 Reaction vessel with aqueous phase
8 Electrostatic charge generator
9 Frequency generator
10 Stroboscope
P pressure control system
S syringe pump

Figure 1: A schematic drawing of the NP production process.

• Differential scanning calorimetry (DSC)

The samples were weighed directly in aluminium crucibles (4- 9 mg) and scanned between 0 and 230 °C at a heating rate of 10 °C/min under nitrogen, using a STARe DSC 1 (Mettler Toledo, USA).

• In vitro dissolution

Freeze-dried NP were redispersed in dissolution medium. At predetermined intervals aliquots were withdrawn and the amount of dissolved celecoxib was determined by HPLC.

RESULTS AND DISCUSSION

The present work demonstrated that high celecoxib-loaded sub 270 nm PLA and PLGA NP can be readily prepared by vibrating nozzle device. The highest drug loading (13 % w/w) was obtained by using 0.1 % w/w PVA and initial ratio celecoxib:Resomer RG 502 of 1:5.

Table 1: Effect of type of polymer, PVA concentration and initial celecoxib:polymer (C:P) ratio on entrapment efficiency (EE) and drug loading (DL) in NP (n=3).

			C:P = 1:5		C:P = 1:10	
Polymer	PLA/ PGA	PVA (%)	DL (%)	EE (%)	DL (%)	EE (%)
RG 752	75/25	0.450	-	-	3.4	70.8
R 202H	100/0	0.450	-	-	3.4	71.0
RG 502	50/50	0.450	6.8	83.9	3.9	89.2
		0.225	9.7	87.3	5.2	84.0
		0.100	13.0	84.4	5.9	82.2

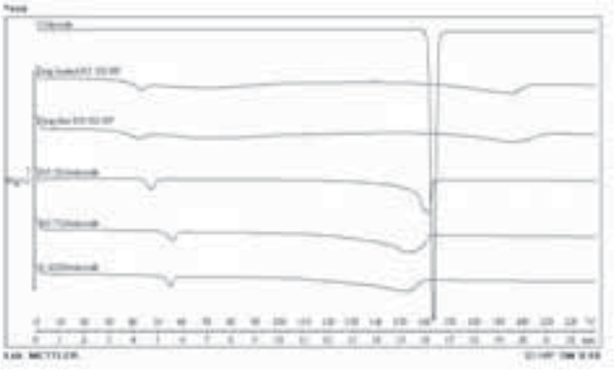


Figure 1: DSC thermograms of celecoxib, binary mixtures of different polymers with celecoxib in ratio 1/1, freeze dried drug-free and celecoxib-loaded Resomer RG 502 NP.

It could be seen from the DSC thermograms of polymer:drug binary mixtures (Fig. 1) that the glass transition temperature of the Resomer polymers decrease with decreasing lactide content. The melting peak of celecoxib in binary mixtures becomes wide and irregularly shaped and is shifted to a lower temperature. This is a consequence of hydrophobic interaction between the polymer and the drug and this interaction decrease with decreasing lactide content in the copolymer composition. Resomer RG 502 seems to have the weakest interactions with the drug. In the thermogram of celecoxib-loaded NP there is no endothermic peak of celecoxib such as in the case of binary mixtures. A broad endothermic peak between 160 and 210 °C corresponds to melting of PVA. Therefore we can assume that celecoxib was molecularly dispersed in the polymeric matrices.

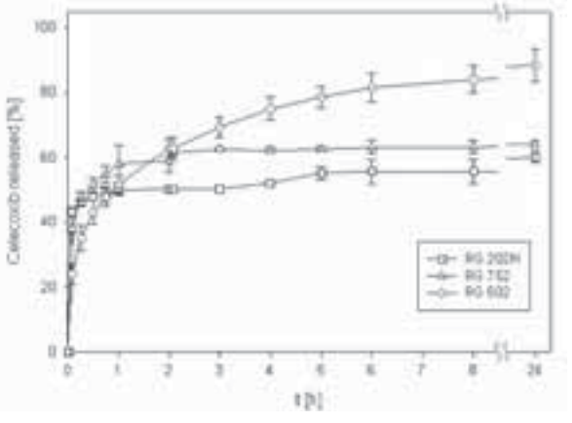


Figure 2: Release profiles of celecoxib from nanoparticles (NP) with different type of polymer.

Due to NP size and consequently large surface to volume ratio, a burst release of ~50 % was observed for all formulations (Fig.2). The drug release rate from NP increased with decreasing lactide content in the matrix composition: celecoxib release was faster from Resomer RG502 (PLA:PGA=50:50) in comparison with Resomer RG752 (PLA:-PGA=75:25) and Resomer 202H (PLA) NP. Drug release was obviously affected by hydrophobic interactions between the drug and the polymer. As it was confirmed by thermal analysis Resomer RG 502 have the

weakest interactions with the drug and therefore this is probably the most important reason for faster drug release.

CONCLUSIONS

In the present investigation the vibrating nozzle device has been used successfully to prepare high celecoxib-loaded PLGA NP. The NP preparationn using vibrating nozzle device can be carried out under mild, nontoxic conditions and can easily be scaled up by using multi-nozzle device. As all parts of the instrument can be sterilized the process can also be carried out under aseptic conditions.

References

1 Leroux et al. 1995, J Pharm Sci 84: 1387.
2 Dong et al. 2004, Biomaterials 25: 2843-2849.
3 Galindo-Rodriguez et al. 2004 Pharm Res 21: 1428-1439.
4 Fessi et al. 1989 Int J Pharm 55: R1–R4.

PO012

Influence of lecithin/chitosan nanoparticles on melatonin permeability

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INTRODUCTION

Indole amide neurohormone melatonin, secreted by the pineal gland, plays an important role in the body's internal time-keeping system. It regulates sleep-wake cycle and other circadian and seasonal rhythms. It is also known for its antioxidant and cytoprotective activity (1). Exogenous melatonin has been used in the treatment of various circadian rhythm disorders, like jet-lag and insomnia. Due to low oral bioavailability and poor water solubility, new routes of administration and delivery systems, are being investigated. In this study, lecithin/chitosan nanoparticles were evaluated in terms of size, surface charge, melatonin entrapment, and their influence on melatonin mucosal permeability.

MATERIALS AND METHODS

Chitosan ChitoClear® FG 95 (Primex, Island); soybean lecithins Lipoid S45, S75 and S100 (a kind gift from Lipoid GmbH, Germany); melatonin (Sigma, USA).

Melatonin-loaded nanoparticle suspensions were obtained by injection of ethanolic lecithin/melatonin solution (lecithin conc. 2,5%, w/v; lecithin/melatonin ratio 5/1,w/w) into water diluted chitosan solution in 0,275 N HCl (1%, w/v) magnetically stirred (900 rpm) (2).

For the comparison, colloidal suspension of lecithin S45 was prepared by injection of ethanolic melatonin/lecithin solution into the pure water. Melatonin-free (empty) nanoparticles were prepared following the same procedure as for melatonin-loaded nanoparticles omitting melatonin. Drug loading was determined using the dialysis technique for separating the non-entrapped drug from nanoparticles.

The average nanoparticles size and zeta-potential was determined by photon correlation spectroscopy and laser Doppler anemometry, respectively (Zetasizer 3000 HS, Malvern Instruments, UK).

The Caco-2 cell line was purchased from ATCC (USA). Cells were maintained in T-75 flasks with MEM (Invitrogen, UK) supplemented with 10% FBS, 1% l-glutamine and 1% nonessential amino acids at 95% humidity and 37 °C in an atmosphere of 5% CO_2.

For the permeability assay, cells were seeded onto the polycarbonate 6-well Transwell® inserts of 3,0 μm mean pore size, 4,67 cm^2 surface area (Corning Costar Inc., NY) at a density of $1x10^5$ cells/well. Transepithelial electrical resistance (TEER) of the monolayer using the epithelial voltohmmeter (EVOM, WPI Inc., USA) was measured to determine the integrity of the monolayers. Permeability studies were carried out in Hank s Balanced Salt Solution (HBSS) pH 7.4 buffer (with 10mM HEPES) at 37 °C. The quantitative determination of melatonin was performed by HPLC (Shimadzu, Japan) analysis. The mobile phase consisted of acetonitrile:water (55:45, v/v).

RESULTS AND DISCUSSION

The nanoparticle samples and their composition are presented in Table 1.

Table 1: Melatonin-loaded lecithin/chitosan (L/C) nanoparticles

Sample	Lecithin type	L/C ratio (w/w)	Drug loading (%)*
C1	S45	1/0	4,9 ± 0,3
C2	S45	20/1	5,3 ± 0,3
C3	S45	10/1	6,4 ± 0,1
A3	S100	10/1	4,3 ± 0,1
B3	S75	10/1	4,5 ± 0,3

*Values are mean ± SD (*n*=3)

Three types of lecithin used differ in the content of lipids that contribute to the negative charge of lecithin, ascending from lecithin S100 to lecithin S45, giving rise to the interaction with positively charged chitosan.

Drug loading, mean diameter and the surface charge of the nanoparticles obtained depended on both, the type of lecithin used and the lecithin to chitosan weight ratio in the preparation (Fig. 1). They all in-

creased with the increase of the chitosan content in the preparation, and also with the negative charge of lecithin, resulting in the stronger interaction between lecithin and chitosan.

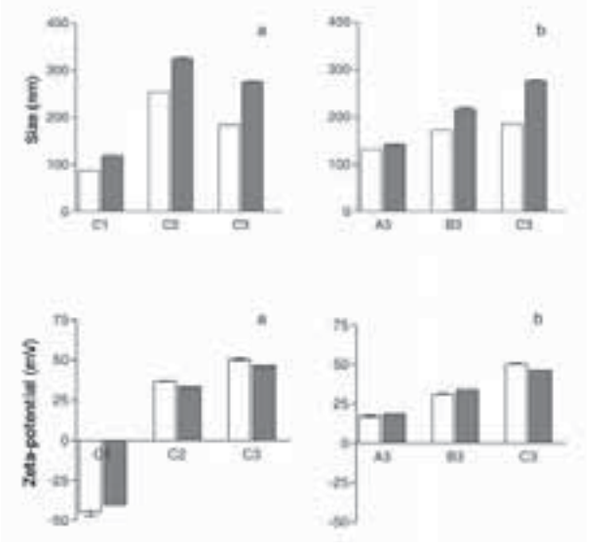

Figure 1: Influence of the chitosan content (a) and type of lecithin (b) on the size and zeta-potential of melatonin-free (□) and melatonin-loaded (■) nanoparticles prepared.

For all nanosystems prepared, the mean diameter of melatonin-loaded nanoparticles was larger than the mean diameter of corresponding melatonin-free nanoparticles (Fig. 1). However, melatonin free and melatonin-loaded nanoparticles did not differ in the zeta-potential.

Permeability studies indicated that incorporation of melatonin into lecithin/chitosan nanoparticles in most cases did not significantly affect its permeability (Table 2.). In case of sample C2 characterised by the largest mean diameter of nanoparticles, melatonin permeability was slightly lower, when compared to the melatonin solution. In case of the sample C3, characterised by the highest drug loading and the highest zeta-potential, melatonin permeability was even slightly improved compared to its permeability from the solution. It can be explained by the fact that chitosan interacted with the cell monolayer opening intercellular tight junctions, thus influencing melatonin permeability.

Table 2: Caco-2 cells permeation of melatonin from aqueous solution (M) and nanoparticle formulations

Sample	P_{app} x 10^5 (cms^{-1})*
M	1,06 ± 0,07
A3	1,09 ± 0,06
B3	1,05 ± 0,10
C1	0,99 ± 0,05
C2	0,80 ± 0,02
C3	1,29 ± 0,02

*Values are mean ± SD (*n*=3)

CONCLUSIONS

Lecithin/chitosan nanoparticles as melatonin delivery system have been successfully developed. Due to their positive surface charge originated from chitosan, they are suitable for mucosal delivery. Melatonin incorporation into lecithin/chitosan nanoparticles did not impair its permeability.

References

1. Mao, S., Chen, J., Wei, Z., Huan, L., and Bi, D., Intranasal administration of melatonin starch microspheres, Int. J. Pharm., 272: 37-43, 2004.
2. Sonvico, F., Cagnani, A., Rossi, A., Motta, S., Di Bari, M. T., Cavatorta, F., Alonso, M. J., Deriu, A., and Colombo, P., Formation of self-organized nanoparticles by lecithin/chitosan ionic interaction, Int. J. Pharm., 324: 67-73, 2006.

PO013

Photostability and antioxidative activity of γ-orizanol included in cyclodextrin-based nanosponges

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INTRODUCTION

Exposure of normal skin to solar radiations produces short-term effects, such as erythema and pigmentation, and long term effects such as carcinogenesis and aging. To protect human skin from the damage a variety of sunscreen lotions have been formulated, to absorb or block UV radiations before they can penetrate into the epidermis. Reactive oxygen species (ROS), deleterious to cells, may be generated from photosensitised reactions. Among cellular targets of ROS, polyunsaturated fatty acids were recently studied and the current hypothesis is that a peroxidative process in cultured human skin cells could be induced either by UVA and UVB [1].

Although synthetic compounds like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG) are widely used as antoxidants, their toxicological aspects have popularized the use of natural antioxidants. γ-Orizanol (GO) extracted from rice bran is a mixture of ferulate esters of triterpene alcohols and is known to be a pow-

erful inhibitor of hydroxyl radical formation [2]. It is employed as antioxidant in food, cosmetic and medical products. Unfortunately, GO is known to be a light sensitive ingredient, thus it could be advantageous to include it in a carrier system to ensure adequate efficacy and stability for this active substance. Nanosponges are innovative carriers consisting of cross-linked cyclodextrins (CD) able to incorporate molecules within their structure. The objective of this research was to investigate the capacity of synthesized nanosponges of β-CD (β-NS) to improve the photostability of GO. Additionally the effect of encapsulation in β-NS on the antilipoperoxidative activity and *in vitro* skin permeability of this molecule was investigated.

EXPERIMENTAL METHODS

• Complex preparation

GO and nanosponges were mixed in water in a ratio 1:1 by weight. The solid dispersion was freeze dried and the obtained powder was used for further work.

• GO diffusion through lipophilic membrane

The permeation behaviour of GO through an artificial silicon membrane was studied.

The donor phase was represented by 20 ml of O/W emulsion (20% octyl octanoate, 6% alkyl polyglucoside, 74% water) containing 1 mM GO, either free or complexed with β-NS. The receiving phase consisted in a mixture of ethanol/water (50/50). The system, sheltered from light, was maintained under stirring (300 rpm) at 25 ± 0.1 °C for 6 hours. At scheduled times of 60 minutes, an aliquot (0.2 ml) of the receiving phase was withdrawn and replaced. The collected samples were analyzed by UV-Vis spectrophotometer to assess GO concentration (detection wavelength at 326 nm).

• Photodegradation studies

GO photodegradation was investigated in O/W emulsion and in 2% hydroxyethylcellulose gel.

The samples (10 ml) were introduced in Pyrex glass containers placed at 10 cm from the UV light source and maintained under continuous stirring. In such conditions the radiation power per surface unit was 6.0×10^{-4} W•cm^{-2} for UVA lamp and 2.4×10^{-4} W•cm^{-2} for UVB lamp. At fixed times the amount on non-degraded GO was assessed spectrophotometrically.

• DPPH• assay

In its radical form, DPPH• adsorbs at 515 nm but upon reduction by an antioxidant its adsorption decreases [3]. The reaction was started by the addition of 100 µl of GO or GO/β-NS (0-250 µM final concentration) to 3.0 ml of DPPH•-saturated water/ethanol (50/50) mixture. Absorbance was recorded after 10 min of magnetic stirring.

• Linoleic acid UV-induced peroxidation

The peroxidation of a lipidic substrate, linoleic acid, was investigated under UVA irradiation in the absence and in the presence of GO, either free or complexed with β-NS. The lipid peroxidation was assessed by malondialdehyde (MDA) determination through a colorimetric reaction with thiobarbituric acid.

The pink coloured complex that absorbs maximally at 535 nm was detected spectrophotometrically [4].

• *In vitro* skin permeation studies

The effect of complexation with β-NS on skin permeation behaviour of GO was studied.

Skin slices from pig ears, obtained freshly from a local slaughterhouse, were set between the donor and the receptor compartments of Franz diffusion cells.

All samples (O/W emulsion containing 1 mM GO pure or complexed with β-NS) were placed in donor compartments (1 ml).

The receptor phases were filled with physiological buffer. At the end of the study (24 h) each skin piece was removed from the device, washed with water, cut up and extracted with methanol to detect GO spectrophotometrically.

RESULTS AND DISCUSSION

Figure 1 shows that membrane diffusion of free GO was faster than GO/β-NS suggesting a certain interaction between the guest molecule and the inclusion agent.

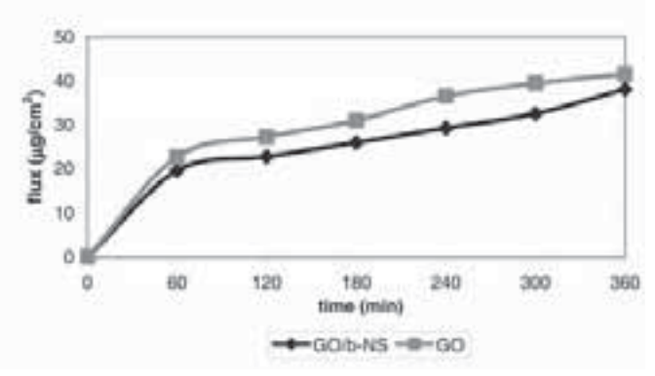

Figure 1: Diffusion profiles of GO, free or complexed with β-NS, from O/W emulsion.

Results of photodegradation studies (Figures 2 and 3) revealed that the inclusion in nanosponges increased the photostability of GO under both UVA and UVB irradiation.

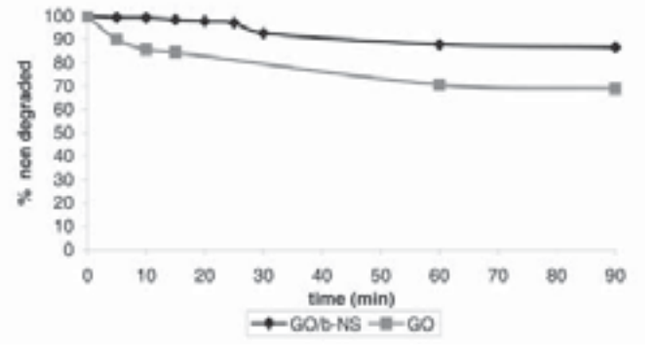

Figure 2: UVA-induced photodegradation trends of GO (1 mM) in O/W emulsion

The trend of photodegradation of GO was found to be related with its initial concentration and with the nature of the vehicle.

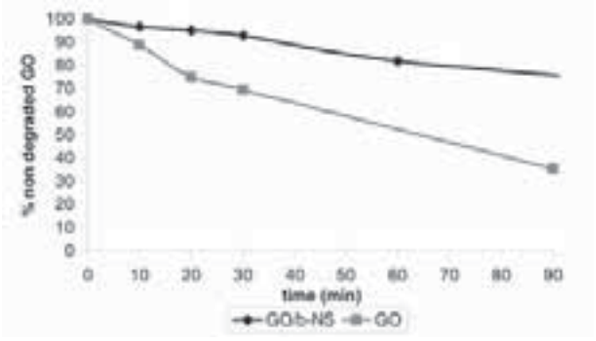

Figure 3: UVB-induced photodegradation trend of GO (0.05 mM) in (2%) hydroxyethylcellulose gel

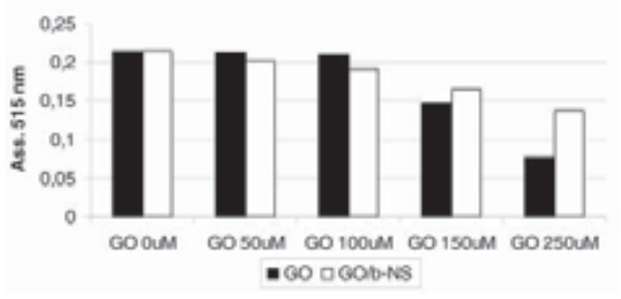

Figure 4: Antiradical activity of GO (■) and of GO/β-NS complex (□).

Figure 4 shows that a higher decline of DPPH• absorbance was observed by increasing GO concentration. The inclusion in -NS slightly affected the antiradical activity of GO but the complex resulted nevertheless efficacious.

Lipoperoxidation and skin permeation values (Table 1) of GO and of GO/β-NS were similar.

Table 1: Antilipoperoxidative activity and skin absorption values for GO and GO/β-NS

Sample	MDA levels (nmol/mg)	Porcine skin (μg/g)
GO	52.6	2.42
GO/ -NS	42.8	2.37

CONCLUSIONS

β-Nanosponges represent a promising carrier system for GO as they are able to improve its photostability without limiting its antioxidative properties and skin permeation.

References

1. Morliere P., Moysan A., Tiroche I. *Free Radical Biology and Medicine* 19(3): 365-371 (1995)
2. Renuka Devi R., Arumughan C. *Food Chem. Toxicol.* 45: 2014-2021 (2007)
3. M. Murias et al., *Biochem. Pharmacol.* 69: 903-912 (2005)
4. Bay B.-H., Lee Y.-K., Tan B. K.-H., Ling E.-A.; *Neuroscience Letters* 277:127-130 (1999).

PO014

Preparation and characterization of oxygen-filled dextran nanobubbles for tissue oxygenation

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INTRODUCTION

Oxygen is the key element in cell metabolism, and its concentration in tissues plays an important role in the efficiency of many biochemical reactions.

In many medical pathologies such as diabetes, burns, bedsores, and wounds, insufficient supply of oxygen to the tissues was observed. A decrease in the oxygen supply from arterial blood to cells of biological tissues substantially reduces the efficacy of medical treatments, increases the risk of infection and scarring, and ultimately leads to tissue necrosis [1].

Moreover oxygen deficiency in tissues of cancerous solid tumors is also a major factor limiting the effectiveness of the therapy.

In a previous work we prepared oxygen-filled chitosan nanobubbles as oxygen delivery system. The in vitro oxygen release was evaluated in hypoxic conditions before and after ultrasound (US) sonications, supporting the hypotesis that the oxygen release from the nanobubbles was enhanced with US. The effect of the oxygen-filled nanobubbles on the cellular expression of the Hypoxia Inducible Factor-1α (HIF-1) was studied on human choriocarcinoma cells showing a reduction of the HIF-1 alpha expression [2].

In the present work, we develop a new formulation of nanobubbles as oxygen delivery system. New oxygen-enriched dextran nanobubbles were prepared using perfluoropentan as core.

To stabilize the nanobubbles, a formulation with polyvinylpyrrolidone (PVP) was also prepared.

These formulations were developed to evaluated their possible application for the treatement of chronic wounds.

EXPERIMENTAL METHODS

Preparation of oxygen-filled nanobubbles

An ethanol solution containing Epikuron® 200 (1% w/v) and palmitic acid (0.3 % w/v) was added to perfluoropentan and filtered water under stirring. The solution was saturated with oxygen up to a gas concentration of 35 mg/l. A 2.7 % w/v dextran solution (M_w dextran=100000) was added dropwise and the mixture was homogenized using an Ultraturrax for 2 minutes, continuing the O_2 purge.

To stabilize the nanobubbles another formulation was prepared adding a polyvinylpyrrolidone solution 5% pH 5.7 (M_w polyvinylpyrrolidone=24000) after the homogenization. Fluorescent dextran nanobubbles were also prepared using dextran labelled with fluorescein isothiocyanate (FITC) to verify the presence of the shell.

The average diameter, polydispersity index and zeta potential of the nanobubbles were determined using a 90 Plus instrument (Brookhaven, Holtsville, NY, USA) at 25 °C. The dextran nanobubbles morphology was evaluated by electronic (TEM) and optical microscopy. Fluorescent microscopy was used with FITC-dextran nanobubbles to evaluate shell thickness and the presence of a gaseous central core.

Biocompatibility assessment

The haemolytic activity of the two formulations was evaluated on human blood after incubation of 25, 50, 100, 150, 200 μl nanobubbles for 90 min at 37 ° C with a solution of red blood cells and then samples were analyzed by spettrofluorimetric method. Preliminary studies of acute toxicity were carried out. 100 μl of dextran nanobubbles acqueous suspension were administrated subcutaneously and intraperitoneally to two groups of four mice.

Determinations of O_2 release

A saline solution (0.9% NaCl) was used to study the oxygen release from nanobubbles .

The oxygen concentration of the solution was reduced with N_2 purge to mimic hypoxic condition at 0.4 mg/l value (severe hypoxia) and 4 mg/l value (medium hypoxia) in two different experiments.

The oxygen concentration in 20 ml of the two solutions was monitored for 10min using an oxymeter (Portamess 913 OXY, Knick) before and after injecting 3 ml of oxygen-carrying dextran nanobubbles to detect the gas release process and measure its kinetics over time.

Stability of dextran nanobubbles

The stability of the two formulation in a saline solution (0.9% NaCl) and in plasma was also studied over time analizing the morphology of nanobubbles by optical microscopy and measuring the nanobubbles dimension with Laser Light Scattering after 5, 10, 15 minutes of incubation at 25, 37, 43 °C.

Oxygen ultrasound delivery

To study the effect of US on nanobubbles an apparatus consists of two compartments separated by a siliconic membrane was used. In the first compartment 70 ml of physiological solution and 10 ml of nanobubbles saturated with oxygen were introduced. In the second compartment 20 ml of physiological solution treated with N_2 up to an oxygen concentration of 0.4 mg/l were placed. The concentration of oxygen in the hypoxic compartment was monitored with an oxymeter for 10 min in the presence and in the absence of US. For the experiment a US probe with a frequency of 2 MHz and an acoustic pressure of 24 bar was used.

RESULTS AND DISCUSSION

The average diameter, the polydispersity of the oxygen-filled dextran nanobubble formulation and the zeta potential are reported in *Table 1.*

Table 1: Characteristic of the two formulations of nanobubbles

FORMULATION	d ± SD (nm)	Polydispersity Index	PZ ± SD (mV)
Dextran	565.7 ± 31.2	0.240	-40.86 ± 1.5
Dextran +PVP 5%	413.5 ± 5.7	0.277	-43.2 ± 1.2

The negative values of the zeta potential confirms the presence of the dextran coating on the surface of the nanobubbles.The final pH of both nanobubble formulations was 6.50. This value is suitable for in vivo administration.

The nanobubble formulations were stable after dilution 1:10 v/v with saline solution (0.9% NaCl) and plasma.

A concentration of the two formulations of 200 μl/ml did not show haemolytic activity after incubation with human blood. No toxic side effects were observed after subcutaneous and intraperitoneal administration of nanobubbles in mice.

A TEM image of dextran nanobubbles is reported in figure 1. The photomicrograph evidenced the presence of a marked dextran shell.

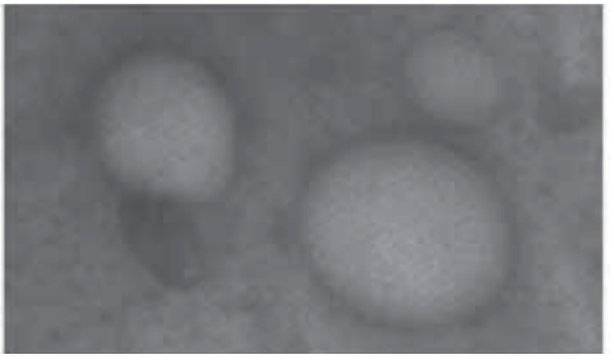

Figure 1: TEM image of dextran nanobubbles (Magnification 27500X)

The increase of oxygen concentration, in hypoxic media after the injection of the two formulations of dextran oxygen-filled nanobubbles is reported in figure 2.

The graph shows that after the addition of nanobubbles the increase in the oxygen concentration was greater at the maximum level of hypoxia (0.4 mg/l) than the one at a medium level of hypoxia (4 mg/l).

In addition, using nanobubbles containing PVP the maximum level of oxygenation was reached faster with nanobubbles containing only dextran at both level of hypoxia considered.

Figure 3 shows the results of oxygen delivery from dextran nanobubbles using US. A marked increase in the oxygen delivery through a siliconic membrane was observed after treatement with US. The PVP nanobubbles showed a similar profile.

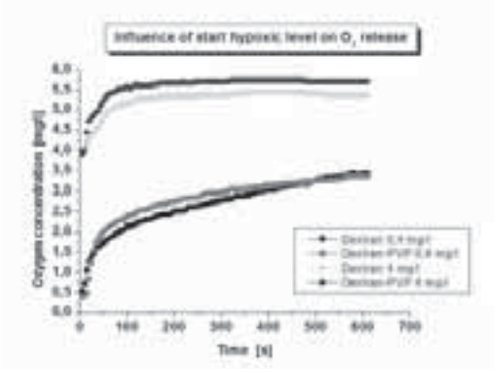

Figure 2: Increase of the oxygenation starting from solutions with maximum (0.4 mg/l) and medium hypoxia (4 mg/l) after addition of dextran nanobubbles and dextran nanobubbles containing PVP

CONCLUSION

Two different formulations of oxygen-filled dextran nanobubbles were prepared. These formulations were found to release oxygen in hypoxic conditions and the gas release could be further enhanced using US.

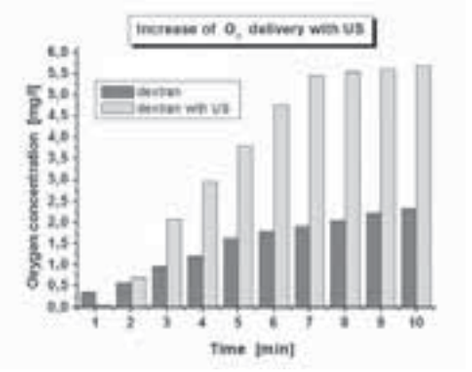

Figure 3: Oxygen delivery from dextran nanobubbles in the presence and in the absence of US through a siliconic membrane

Preliminary studies did not show toxic side effects after *in vivo* administration.

ACKNOWLEDGMENTS

The authors wolud like to thank the support of PRIN 2006 Italian Ministry of University and Ricerca Sanitaria Finalizzata Project 2006 of Regione Piemonte.

References

1. M. M. Asimov, A. N. Korolevich, E. É. Kostantinova. Kinetics of oxygenation of skin tissue exposed to low-intensity laser radiation, J. of applied spectroscopy (2007)
2. R.Cavalli, A. Rolfo, S. Balbis, A. Bisazza, C. Trotta, D. Madonnaripa, I. Caniggia, C. Guiot. Chitosan nanobubbles as oxygen delivery system for the treatement of hypoxic tissues, CRS 2008.

PO015

Effective topical delivery of antifungal drug by nonionic surfactant based vescicles (niosomes)

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INTRODUCTION

Niosomes are unilamellar or multilamellar vesicles formed from synthetic non-ionic surfactants of the alkyl or di-alkyl poly glycerol ether class offering an alternative to liposome as drug carriers (Uchegbu and Vyas, 1998). There are various methods reported in literature for the formulation of niosomes such as Ether injection method (Bangham et al., 1976), Lipid Film formation or hand shaking method (Baille et al., 1985, Chandraprakash et al., 1983, Rogerson et al., 1987, Azmin et al., 1985), Reverse phase evoparation (Almira et al., 2001). The method of preparation of niosome is based on liposome technology. Fluconazole (FLZ) was chosen as antifungal agent has been effective in the treatment of infections caused by *Candida albicans* and dermatophytes.

EXPERIMENTAL

• Preparation of Niosomes

Niosomes formulations were prepared by Lipid Film hydration technique. Drug, non-ionic surfactant and cholesterol were weighted as indicated in table 1 and dissolved in chloroform/ methanol system (2:1) in a 100 ml round bottom flask. The solvent mixture was evaporated at a temperature (25 ± 2°C) and the flask rotated at 100 rpm until a smooth, dry lipid film was obtained. The film was hydrated with 8-10 ml of PBS pH 7.4 for 45 min at room temperature (25 ± 2°C) With gentle shaking the niosomal suspension was further hydrated at 2-8°C for 24 hr.

CHARACTERIZATION OF NIOSOMES

• Entrapment Efficiency

Determination of FLZ entrapment efficiency in niosomes was performed by a direct method given by Ruckmani et al, 2000.The concentration of FLZ in 0.1N HCl was determined spectrophotometrically at 261 nm using a UV-visible spectrophotometer (Shimadzu 1700, Japan).

• In Vitro Drug Release Profile

In Vitro drug release from the various niosomes systems was performed in 0.1 N HCl using treated cellophane membrane mounted on one end of dialysis tube. The sample being withdrawn was assayed spectrophotometrically after making proper dilution.

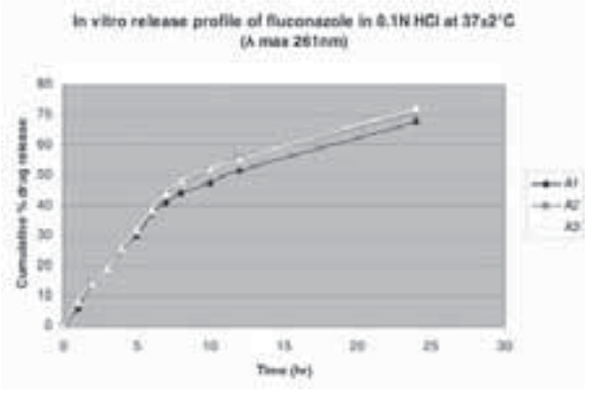

Figure 1: In vitro release profile of Fluconazole in 0.1 N HCl (Span 20)

• In vitro permeation study

Franz (vertical) diffusion cell was used to verify the access of FLZ from niosomes. The Wister rat (7–9 weeks old) skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. The donor compartment was filled with the niosome formulation. A 20 ml aliquot of 40%:60% (v:v) ethanol:pH 7.4 phosphate buffer was used as receptor medium to preserve a sink condition. At suitable intervals 200 ml aliquots of the receptor medium were withdrawn and instantly replaced by an equal volume of fresh receptor solution. The samples were analyzed by UV spectrophotometric method.

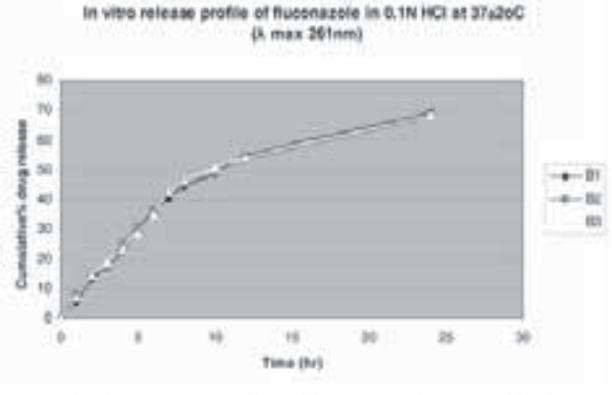

Figure 2: In vitro release profile of Fluconazole in 0.1 N HCl (Span 60)

• *In Vitro* Antimycotic Studies

The antimycotic studies were conducted as per method described by using agar-cup method and *Candida albicans* as a test organism. The method was applied on all niosomes formulation, the values for zone of inhibition were recorded and the observations were compared with marketed formulation.

RESULTS AND DISCUSSION

The mean particle diameters of LFH niosomes composed of Span 20 and cholesterol in a 1:2 molar ratios were 3.81 μm. While the mean particle diameters of LFH niosomes composed of Span 60 and cholesterol in a 1:2 molar ratio were 3.58 m. The results has shown that the effect of cholesterol concentration, in case of span 20 as well as span 60, on the vesicles size, is minimal and no significant differences in their size were observed. The effect of surfactant type, on the vesicle size shows that the niosomal vesicles prepared by using Span 20 is larger in size than niosomes prepared using Span 60. Data shows that increasing the incorporation of cholesterol into niosomes was found to increase the encapsulation efficiency of FLZ but only up to an optimum concentration of cholesterol. The percentage entrapment efficiency of FLZ in niosomes increased from 35.29 % to 40.11 %. LPH niosomes are composed of Span 20 and cholesterol in a 1:1and 1:2 molar ratios. Also the percentage entrapment efficiency of FLZ in niosomes increased from 27.83% to 30.06 % for LPH niosomes composed of Span 60 and cholesterol in a 1:1 and 1:2 molar ratios. Further increase of cholesterol content, reaching a 2:1 molar ratio, for niosomes composed of Span 20 or Span 60 and cholesterol reduced the entrapment efficiency for LPH niosomes. This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bilayered structure leading to decreased drug entrapment efficiencies for niosomes prepared using Span 20 were superior to those prepared using Span 60. From the results of *In Vitro* release study of FLZ from various LFH niosomal formulations, it is concluded that the increase of surfactant molar ratio from 1:1 to 1:2 markedly reduced the efflux of the drug, which may be in accordance with its membrane stabilizing ability. By reviewing the data in figure 1 the release of drug was 67%, 70% and 72% from Span 20 formulations A1, A2 and A3 respectively. From the data of figure 2 the release of drug was 69%, 68% and 68% from Span 60 formulations B1, B2 and B3 respectively. It was found that values for *In vitro* Antimycotic activity of A2 formula were significantly more from other respective formulations. This indicates that formulations comprising Span 20 effectively delivered drug at concentration exist in A2 formulation. Hence it may be concluded that the formulation A2 exhibited better antimicrobial activity in comparison to respective formulations.

CONCLUSION

The effect of varying cholesterol ratio has direct effect on the integrity of vesicle membrane. Entrapment efficiency increases with increasing cholesterol concentration but only up to a certain limit this may be because the cholesterol has the ability to cement the leaking space in bilayer membranes, but after a definite concentration it results in leaky vesicles. While the effect of varying surfactant, on the vesicle size shows that the niosomal vesicles prepared by using Span 20 is larger in size than niosomes prepared using Span 60. *In Vitro* release of FLZ from niosomes system was compared with marketed formulation. It was concluded that release behavior of A2 formulation of Span 20 was better and equivalent to that of marketed preparation. Zone values of nio-

somes formulation were estimated using agar-cup method and *Candida albicans* as a test organism.

References

1. Uchegbu, I. F., Vyas, S. P., (1998). Non-ionic surfactant based vesicles (niosomes) in drug delivery. *Int. J. Pharm.*, 172, 33–70.
2. Baillie, A.J., Coombs, G.H., Dolan, T.F., Laurie, J., (1986). Non-ionic surfactant vesicles, niosomes, as a delivery system for the anti-leishmanial drug, sodium stibogluconate. *J. Pharm. Pharmacol.*, 38, 502– 506.
3. Chandraprakash, K.S., Udupa, N., Umadevi, P., Pillai, G.K., (1993). Effect of macrophage activation on plasma disposition of niosomal 3H-Methotrexate in sarcoma-180 bearing mice. *J. Drug Target.*, 1, 143–145.
4. Rogerson, A., Cummings, J., Florence, A.T., (1987). Adriamycin-loaded niosomes: drug entrapment, stability and release. *J. Microencap.*, 4, 321–328.
5. Azmin, M.N., Florence, A.T., Handjani-Vila, R.M., Stuart, J.F.B., Vanlerberghe, G., Whittaker, J.S., (1985). The effect of non-ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice. *J. Pharm.Pharmacol.*, 37, 237–242.
6. Ruckmani, K., Jayakar, B., Ghosal, S.K., (2000). Nonionic surfactant vesicles (niosomes) of cytarabine hydrochloride for effective treatment of leukemia: encapsulation, storage and in vitro release. *Drug Dev. Ind. Pharm.*, 26, 217–222.

PO016

Importance of resveratrol loaded in liposomes for cell survival under stress conditions

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INTRODUCTION

Several studies have been demonstrated that polyphenolic compound resveratrol (RSV) in a first line inhibits production of reactive oxygen species (ROS) and lipid peroxidation. Furthermore, interactions of RSV with receptors and enzymes give rise also to other biological effects, such as suppression of growth in tumour cells, down-regulation of proinflammatory mediators, cell cycle regulation and induction of differentiation. To summarize, investigations of RSV biological effects evidenced its dual action, as it participates in prosurvival as well as in prodeath cellular mechanisms, depending on cellular conditions, specific cell molecular settings and the concentration used [1]. Additionally, low solubility and physical instability make RSV vehicolation a real technological and medical challenge [2].

Our aim was to determine the effect of different RSV doses on metabolic activity of HEK 293 cells. Results of a well known cytotoxicity test, MTS assay, were compared with results of RSV reducing capacities, obtained by EPR technique. In addition, RSV-loaded liposomes (LIP-RSV) were prepared to allow controlled release of RSV over a prolonged period of time and to protect RSV from light or other degradative processes. Effect of LIP-RSV on cell metabolic activity was determined and compared with results obtained from RSV alone. Moreover, the ability of RSV to cope with stress situation (UV-B light) was also checked.

EXPERIMENTAL METHODS

• ***Preparation and characterization of LIP-RSV and RSV dispersions.***

LIP-RSV were prepared by sonication and subsequent extrusion (LiposoFast™extruder) of cholesterol, dicetyl phosphate, phosphatidylcholine and RSV in Tris-HCl. The size, polydispersity index (PI) and zeta potential were determined by Zetasizer nanoZS (Malvern Instrument). The incorporation efficiency of RSV-loaded liposomes was determined at 306 nm using an Agilent 1100 Series HPLC System (C18; flow rate: 0.8 ml/min; mobile phase: (methanol, acetonitrile, water, acetic acid) (75:22.5:2.4:0.1, v/v)).

Stock solution of RSV in ethanol (100 mM) was also prepared and diluted with the culture medium before experiments.

• ***Cell culture and treatment.***

Human embryonic kidneys cells (HEK293, ATCC), cultured in supplemented DMEM, were treated for 24 h with RSV dispersion or with LIP-RSV. Both test samples were investigated at two different concentrations of RSV, 10 and 100 μM. Control cells were incubated with DMEM. Stress conditions were simulated by irradiating the cells at 280-320 nm (UV lamp, Spectronics) for one or three hours.

• ***MTS assay.***

The metabolic activity of treated cells was evaluated in vitro using the Cell titer 96® Aqueous One Solution Cell Proliferation Assay (Promega). The results were expressed as a fraction of non-irradiated control cells.

• ***EPR.***

The determination of cell metabolic activity was evaluated also by measuring the capability of the cells to reduce the lipophilic nitroxidic spin probe MeFASL-(10,3) into a nonparamagnetic compound. Decrease of EPR spectral intensity was followed by X-band EPR spec-

trometer (BrukerESP300; room temperature; microwave frequency 9.59 GHz; power 11 mW; modulation frequency 100 kHz; amplitude 0.2 mT).

RESULTS

Evaluation of protective potential of RSV

In non-irradiated cells free RSV at 10 μM was well accepted by the cells, while RSV at 100 μM caused an arrest in cell proliferation (Fig. 1). In contrast to non-radiated cells, a drop of metabolic activity appeared when cells are exposed to stress conditions. However, RSV at 10 μM proved again to have a preventive effect if compared with RSV at 100 μM.

The metabolic activity of treated cells, acquired by EPR technique via reduction kinetics of the spin label, is represented in a form of rate constants (1/s), calculated from the slope of the kinetic curves. Obtained constants (1/s) are: for the non-irradiated cells [4.94×10^{-2} in control, 6.26×10^{-2} in RSV 10 μM and 2.93×10^{-2} in RSV 100 μM] and for irradiated cells [2.64×10^{-2} in control, 5.25×10^{-2} in RSV 10 μM and 1.74×10^{-2} in RSV 100 μM]. A faster reduction, which indicated higher cell vitality, was observed in non-irradiated samples. In general, RSV at 10 μM caused the fastest nitroxide reduction, whereas RSV at 100 μM retarded cellular activity. UV radiation, as a stress factor, diminishes cell vitality for approximately two times, as proved by EPR and MTS assay.

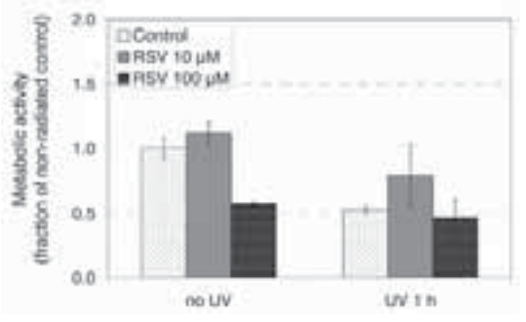

Figure 1: A dose-dependent decrease of HEK293 cell metabolic activity of after 24 h incubation with RSV at 10 or 100 mM and subsequent exposure to (non)stress condition.

Table 1: Correlation between cell metabolic activity determined by MTS and EPR technique. Cells were treated with RSV solution at 10 or 100 mM for 24 h and then exposed to (non)radiation.

Condition	no UV		UV 1h	
Metabolic activity	MTS	EPR	MTS	EPR
Control	1.00	1.00	0.56	0.53
RSV 10 μM	1.11	1.27	0.84	1.06
RSV 100 μM	0.57	0.59	0.50	0.35

For the characterization of radical decay, the term relative redox capacity (RRC) was used [3]. It is defined as the ratio of the kinetic constant of the sample always regarding to non-irradiated control sample. This parameter enables a comparison between results from MTS assay and EPR technique. For each method separately, all calculations were made referring to the results from non-treated and non-irradiated cells (control). Results are collected in Table 1.

The RRC values quite well coincided with results obtained by MTS assay, especially in non-irradiated samples. However, a trend is evident in each group, in non-irradiated as also in irradiated samples, indicating a good correlation between the two techniques.

Liposomes ameliorate effect of RSV

In order to improve RSV stability and to deliver it to the cells, RSV-loaded liposomes (LIP-RSV) were prepared, showing a size of ~100 nm, a P.I. of ~0.2, a negative surface charge and an entrapment efficiency of ~75%.

The effect of LIP-RSV was expressed different regarding the treatment conditions (Fig. 2). Interestingly, in non-irradiated cells treated with LIP-RSV, a significant increase of the metabolic activity was seen, irrespective of RSV concentration. Furthermore, the elimination of the cytotoxicity of RSV at 100 μM was observed. However, RSV at 10 μM incorporated into liposomes lost their beneficial effect when used on irradiated cells. Thus the cells need of antioxidants is not satisfied and metabolic activity is decreasing. On the contrary, LIP-RSV at 100 μM promoted metabolic activity under stress conditions. Regarding inhibition of cell metabolic activity, observed in the presence of RSV alone at 100 μM (Fig. 1), preventive effect of LIP-RSV at 100 μM can be achieved by controlled (slow) release of incorporated RSV.

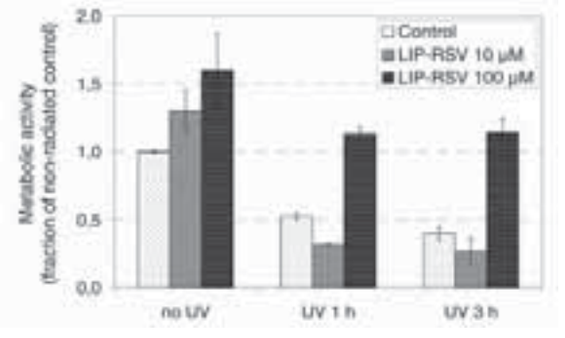

Figure 2: Metabolic activity of HEK293 cells after incubation for 24 h with LIP-RSV (10 mM or 100 mM) and subsequent exposure to radiation for 1 h or 3 h.

CONCLUSION

RSV influences the metabolic activity of HEK 293 cells in a dose-dependent manner, as determined by MTS and EPR assay. Moreover, our results pointed out the importance of RSV in the protection of normal cells against destructive processes of stress conditions. However, regarding concentration-dependence, the effect of RSV is unfortunately two-edged. Thus, loading of RSV into liposomes proved to be cell-beneficial. Slow release of RSV from liposomes prevented the cells from a massive cellular distribution of the drug. Amount of released RSV is just enough to cope with the oxidative stress and thus cell ability to survive is increased.

Reference

1. D. Venkat Ratnam et al, J.Control. Release **113** 189–207 (2006)
2. C. Caddeo et al, submitted for publication
3. M.Budai et al, J. Photochem. Photobiol., B **77** 27–38 (2004)

PO017

Safety or potential cytotoxicity of solid lipid nanoparticles

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INTRODUCTION

Besides new and fascinating properties of nanosized carriers, toxicological problems have to be faced and solved. Design of solid lipid nanoparticles (SLN) that are stable, non-inclined to aggregation and of desired size, is achieved by using surface active compounds, like stabilizers. Since the stabilizers are present on the particle surface, they not only affect its properties but are of considerable importance for the interaction with cells and may as such increase the risk of unwanted or even toxic effects [1]. A small number of basic *in vitro* cell culture studies have been performed to evaluate stabilizers toxicity. Widely used oligomeric non-ionic surface active stabilizers are also Lutrol and Tyloxapol. Their applicability in the area of SLN has been stopped on the level of preparation- and stability-optimization, while the cellular response on their presence has not attracted attentions [2].

The aim of this work was to examine the metabolic activity and morphological changes of cells as the consequence of stabilizers presence alone or adsorbed at nanoparticles surface. A rate of cell detaching, triggered by used stabilizer, was investigated by EPR method and analysis of its effects on cell cycle using flow cytometry.

EXPERIMENTAL METHODS

• Preparation of nanoparticles dispersions and stabilizer solutions.

Ingredients used for SLN, Compritol 888ATO (10.0 %, w/w) and Phospholipon 80H (3.0 %, w/w), were melted at approximately 10°C above their melting point, poured into a hot aqueous solution of steric stabilizer (1 %, w/w) (Tyloxapol, for SLN-Tyl, and Lutrol, for SLN-Lut) and stirred with homogenizer for 10 min at 20500 rpm (Ultra turrax T 25, Janke and Kunkel, Staufen, Germany). Particle size and zeta potential of nanoparticles were determined by photon correlation spectroscopy using Malvern Zetasizer 3000 (UK). Solutions of steric stabilizers (Tyloxapol, Lutrol) were prepared in PBS at the same concentration as present in the SLN dispersion.

• Cell culture and treatment.

Human embryonic kidney cells (HEK 293, ATCC), cultured in supplemented DMEM, were treated with nanoparticles dispersion (1000 μg/ml) or stabilizers solutions (100 μg/ml) for defined time period. Control cells were incubated with PBS.

• MTS assay.

The effect of test dispersions on the metabolic activity of cells was evaluated *in vitro* using the Cell titer 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, US). The results were expressed as a fraction of the absorbance of untreated cells.

• Cell morphology.

Cell growth was determined using an inverted phase-contrast microscope (Olympus CKX41, Tokyo, Japan) and a digital camera (Olympus C-7070).

• Flow cytometry.

Content of DNA in treated HEK 293 cells was determined via quantification of propidium iodide fluorescence intensity with a FACSCalibur cytometer (BD Biosciences, San Diego, USA).

• EPR.

Undamaged cell membrane can incorporate lipophilic spin probe, like paramagnetic MeFASL- (10,3) [3] which intensity can be accompanied by X-band EPR spectrometer (BrukerESP300; room temperature; microwave frequency 9.59 GHz; power 11 mW; modulation frequency 100 kHz, and amplitude 0.2 mT). Because medium with detached cells is removed before spin labeling, obtained signal reflects quantity of the cells that stayed attached with undamaged membrane.

RESULTS

Two formulations of SLN were prepared that contained the same lipid core composed of Compritol and Phospholipon but different non-ionic steric stabilizers. Thus Lutrol was used for SLN-Lut but Tyloxapol for SLN-Tyl. Cell morphology, which was observed by photographing treated cell cultures, was not altered when SLN-Lut were present. Opposite, SLN-Tyl induced detachment of HEK 293 cells (Fig. 1 below). In accordance, also metabolic activity was significantly changed only in SLN-Tyl treated cells (Fig. 1 above), while SLN-Lut did not show such kind of influences.

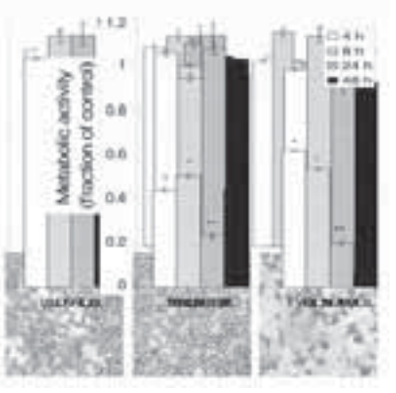

*Figure 1: SLN-Tyl (1000 μg/ml) but not SLN-Lut (1000 g/ml) decreased metabolic activity after designated incubation time (above) and in 24 h induced detachment of HEK 293 cells as well (below). (*P<0.05 and **P<0.001)*

Since the stabilizers are adsorbed on the particle surface, they not only affect its properties but are of considerable importance for interaction with cells. Thus, further investigations were pointed on individual SLN stabilizers. The exposure of HEK 293 cells to Lutrol had no significant

effect on proliferation (Fig. 2 above). In contrast, incubation with Tyloxapol solution resulted in a significant decrease of metabolic activity. Again, cells treated with Lutrol looked similar to the untreated healthy cells (control), while Tyloxapol presence caused changed cell morphology in a form of enormous detachment (Fig. 2 below).

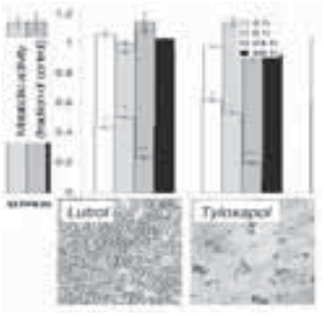

*Figure 2: Opposite to Lutrol alone (100 μg/ml), Tyloxapol on itself (100 μg/ml) time-dependently diminished metabolic activity (above) and in 24 h induced enormous detachment of HEK 293 cells (below). (*P<0.05 and **P<0.001)*

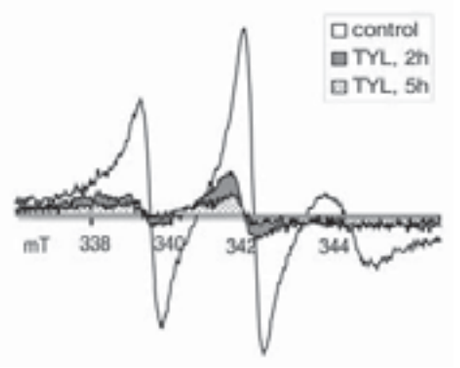

Figure 3: Diminishes of intensity of MeFASL(10,3) EPR spectra loaded in membrane of HEK 293 cells after 2 or 5 h incubation with Tyloxapol solution (100 μg/ml) in comparison with control cells.

A cytotoxic effect of Tyloxapol on cell membrane integrity was confirmed by EPR technique. While paramagnetic compound (MeFASL(10,3)) is adopted by many cell membranes in control sample, the harmful effect of Tyloxapol is so vigorous, that number of the cell membranes capable to load MeFASL(10,3) fall almost to zero after 5 h of incubation (Fig. 3).

The effect of stabilizers on genetic material showed that 48 h incubation with stabilizer Lutrol caused relatively unaltered cell cycle that was comparable to the control (Fig. 4). Pronounced alterations in cell cycle profile were observed in case of Tyloxapol alone, where a significant rise in the number of dead cells with fragmented DNA was observed (Fig. 4; encircled).

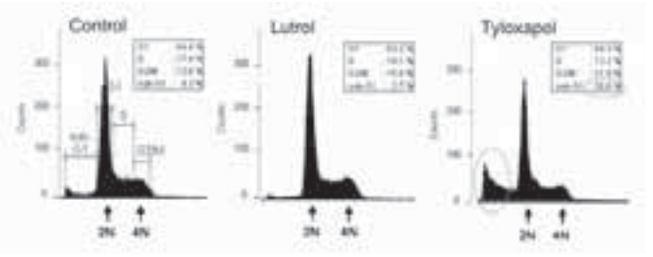

Figure 4: Significant increase of fragmented DNA after 48 h incubation the cells with Tyloxapol (100 μg/ml) (encircled), while similar cell cycles were obtained in case of control cells and cells treated with Lutrol (100 μg/ml) for 48 h. 2N: diploid cells (G1 phase); 4N: tetraploid cells (G2/M phase).

CONCLUSION

Deteriorative cell alterations were detected only in the case of Tyloxapol, either present alone or adsorbed on SLN surface. However, a drop of metabolic activity and cell detaching were even more evident in the case of Tyloxapol alone. Rapid damages of membrane were proved by EPR. Moreover, longer incubation time with Tyloxapol exposed augmented cell dying that was seen in changes of genetic material on cell cycle profile. These findings clearly point out that a careful selection of stabilizer is a crucial step in designing SLN with minimal cytotoxic properties.

References

1. K. Unfried et al, Nanotoxicology **1** 52-71 (2007)
2. J. Kristl et al, submitted for publication
3. J. Kristl et al., Int. J. Pharm. **256** 133-40 (2003)

PO018

The use of immuno-nanoparticles for impairement of intracellular tumor associated proteolytic activity in invasive breast tumor cells

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Tumour associated proteases represent promising targets for anti-tumour therapy. However, the delivery of anti-tumour agents, in particular protein molecules, to tumour proteases is rather difficult without using delivery systems, such as polymeric nanoparticles. Specific delivery of drug-incorporated nanoparticles can be achieved via active targeting by introducing ligands that are recognised uniquely by receptors or certain other proteins expressed on tumour cells. Nanoparticle system is especially efficient for targeting of intracellular lysosomal proteins, including cathepsins, which are known to be involved in processes of tumour progression.

To inactivate lysosomal cysteine protease cathepsin B in tumour cells, we developed poly(lactide-co-glycolide) PLGA nanoparticles, incorporating active cysteine protease inhibitor cystatin and labelled with monoclonal antibody, specific for cytokeratins expressed on the surface of breast epithelial tumour cells. First, cystatin was incorporated into PLGA polymeric matrix and then, the antibody was adsorbed onto the cystatin loaded nanoparticles. The new delivery system was tested on co-cultures of invasive breast epithelial MCF-10A neoT cells, enterocytic Caco-2 cells or differentiated monocyte/macrophage U-937 cells. By using fluorescent microscopy and flow cytometry we showed that the antibody labeled cystatin-loaded nanoparticles solely bound to MCF-10A neoT cells. Moreover, they were rapidly internalized and reduced proteolytical activity of cathepsin B.

Our results show that our new nanoparticulate delivery system enables active targeting of breast cancer tumour cells and efficient intracellular inhibition of tumour associated proteolytic activity.

PO019

Development of papain microcapsule using Na-alginate and study on the *in vitro* and *ex vivo* release of protein from the microcapsule

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INTRODUCTION

The common delivery system of protein therapeutic is via parenteral route due to the poor characteristic of this molecule like poor chemical and physical stability. Its instability in the GI environment lead to the difficulty of oral administration of protein therapeutic. Microencapsulation technique is one approches has been developed to formulate protein therapeutic with improved stability and acceptability. The aim of this study is to develop formulation of protein therapeutic for oral route of administration. Papain was used as a model of protein.

METHODS

Microencapsulation of papain was prepared with $CaCl_2$ crosslinked sodium alginate. Various factors such as stirring speed, sodium alginate concentration (1 % and 1.5 %), and $CaCl_2$ concentration (0.1 M and 0.15 M) were studied. The characteristic microcapsule included particle size and particle size distribution, particle morphology, percentage of entrapment, and the release profile of papain from microcapsule in vitro and ex vivo.

RESULTS

Various factors studied during development of microencapsulation process influenced the characteristic of papain microcapsule. The optimum formula resulted in good characteristic of papain microcapsule was using 1% sodium alginate, 0.15 M $CaCl_2$ and stirring speed of 300 rpm. This optimum formula resulted in high percentage of entrapment as >90%. The release study of papain from microcapsule showed that alginate-crosslinked retained the release of protein in acidic environment both in vitro and ex vivo. In contrast, papain was highly released from microcapsule (>90%) in high artificial intestinal environment for 3 hours in vitro and >90% for 12 hours ex vivo.

CONCLUSION

Chemical reaction between sodium alginate and $CaCl_2$ resulted in alginate cross-linked is capable to encapsulate protein. $CaCl_2$-crosslinked alginate microcapsule retained the release of papain in simulation of gastric fluid and released the protein in intestinal environment both in vitro and ex vivo. We conclude alginate crosslinked microcapsule is a promising approach to formulate protein for oral route of administration.

PO020

Biodegradable PLGA microparticles as a delivery system for protein: effect of molecular weight of PLGA on the microparticle characteristics

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INTRODUCTION

For the past two decades, polymers are being extensively used in drug delivery systems. Polymers by virtue of their ability to sustain the drug release over long periods of time and provide steady plasma concentration may reduce the total dose and some adverse reactions. All of these attributes have made the polymers a requisite part of any drug delivery system, be it a conventional or a novel delivery system. A number of polymers have been exploited for formulating drug delivery systems, but poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers poly(lactide-co-glycolide) (PLGA) have been extensively employed because of their biocompatibility, biodegradability and versatile degradation kinetics [1].

Among many drug delivery systems prepared PLGA microparticles are the most popular form due to manufacturing advantages as well as ease of administration. Various properties of PLGAs such as molecular weight and its distribution, the copolymer composition and the cristallinity effect characteristics of microparticles.

The primary objective of this study was to evaluate the effect of molecular weight (Mw) of PLGA on the characteristics of microparticles prepared by double emulsion technique. Ovalbumin was used in this study as a model protein.

EXPERIMENTAL METHODS

• Preparation of PLGA microparticles

Microparticles were prepared according to a w/o/w double-emulsion method [2, 3]. Briefly, 200 mg of PLGA polymer was dissolved in 1 ml dichloromethane, while ovalbumin (5 mg) was dissolved in 200 µl distilled water. Then, the ovalbumin solution was mixed to the organic phase containing the polymer to obtain a primary w/o emulsion. The emulsification was carried out for 2 min by a homogenizer (Ultraturrax T-25 homogenizer) at 13 500 rpm. This primary emulsion slowly injected into 4 ml of 6% PVA water solution and homogenized at 8000 rpm for 5 min to form the final w/o/w double emulsion. For solvent extraction, the final emulsion was diluted in 50 ml of distilled water and stirred with a mechanic stirrer for about 2 h. The resulting microparticles were filtered by a cellulose acetate membrane filter, washed with distilled water and freeze-dried overnight. The dried microspheres were stored in a sealed glass vial and placed in a desiccator at +4 °C.

• Particle shape and size

To determining shape and size of microparticles, they were dispersed in a droplet of water, directly on a slide and observed under a polarizing microscope (Leica DM4500 P).

• Determination of encapsulation efficiency

Ovalbumin content in the microparticles was determined by an extraction method. A certain amount of dried microparticles was dissolved in dichloromethane. After centrifugation and removal of the polymer solution, remaining ovalbumin pellet was dissolved in 2 ml of phosphate buffer saline (pH 7.4). The ovalbumin concentration was determined by the Micro-BCA protein assay. The absorbance of each sample was read on a microplate reader at a test wavelength of 570 nm. The loading efficiency was determined using following equation:

$$\% \text{ encapsulating efficiency} = \frac{La}{Lt} \times 100$$

Where La is the amount of protein embedded in microparticles and Lt is the theoretical amount of protein incorporated into microparticles. Placebo microspheres (without ovalbumin) were used as control. All determinations were conducted in triplicate.

• In vitro dissolution studies

A certain amount of dried microparticles was suspended in 1,5 ml of PBS (pH 7.4, 0.02% sodium azide, 0.15% SDS) and was then incubated under mild stirring at 37 °C. At each sampling time, the supernatant was withdrawn and replaced with the same volume of fresh PBS solution. The measurement ovalbumin content in the supernatant was performed at 570 nm by Micro-BCA protein assay. Analyses were conducted in triplicate.

RESULTS AND DISCUSSION

Two different PLGA samples (Resomer 502 and Resomer 504), either alone or in combinations, were used to prepare microparticles. The particle size of microparticles prepared Resomer 504 increased with increase in molecular weight from 12,000 to 48,000 Da (Figure 1). One possible reason could be the increase in viscosity of the polymer solutions with increasing molecular weight, thereby posing difficulty in stirring them into smaller emulsion droplets in contrast to the low molecular weight polymers because of lower stirring efficiency at the same agitation speed.

The efficiency of encapsulation increased significantly when a mixture of 1 part Resomer 502 and 1 part Resomer 504 was used to prepare the microparticles. Blending of PLGA showed a significant effect on the efficiency of encapsulation. The efficiency of encapsulation of this batch was 46,31%, whereas the efficiency of encapsulations was 14,92 and 27,78%, respectively, when the microparticles were prepared with 100% Resomer 502 or 100% Resomer 504.

Figure 2 shows the dissolution profiles of ovalbumin from the microparticles up to 42 days. The burst release was measured by determining the initial drug release at 4 h. At microparticles prepared with 100% Resomer 504, the initial release as well as the release rate increased with increasing the drug loading. However, although higher encapsulation efficiency was obtained with mixture of polymer, initial release was very low at this formulation (1,7%).

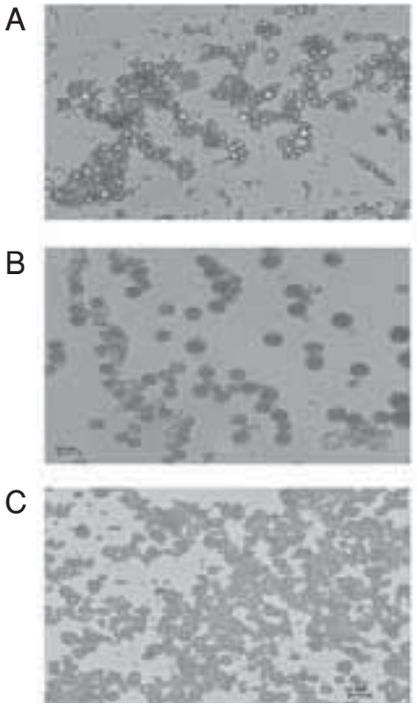

Figure 1: Optical microscopy photographs of PLGA microparticles prepared with A)100% Resomer 502 B)100% Resomer 504 C)mixture of 1 part Resomer 502 and 1 part Resomer 504.

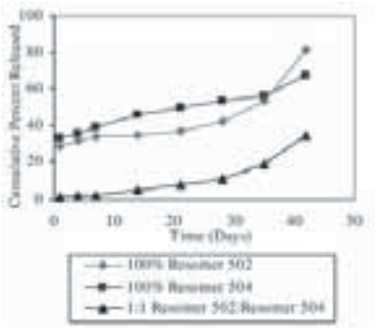

Figure 2: Release profiles of ovalbumin PLGA microparticles in PBS at pH 7.4 and 37 C.

CONCLUSION

PLGA polymer has been used for more than two decades for the development of controlled release microparticles of drugs. A wide variety of PLGA polymers are currently available commercially to achieve desired physicochemical characteristics of these microparticles.

In our present study, efficiency of encapsulation increased significantly when Resomer 504 was mixed with Resomer 502 at a ratio of 1:1. Moreover, very low initial burst release was obtained with mixture of polymer.

References

1. Mittal G., Sahana D.K., Bhardwaj V., Ravi Kumar M.N.V. Estradiol loaded PLGA nanoparticles for oral administration: Effect of polymer molecular weight and copolymer composition on release behavior in vitro and in vivo. Journal of Controlled Release 119:77-85 (2007).
2. Giovagnoli S., Blasi P., Schoubben A., Rossi C., Ricci M. Preparation of large porous biodegradable microspheres by using simple double-emulsion method for capreomycin sulfate pulmonary delivery. International Journal of Pharmaceutics 333: 103-111 (2007).
3. Devrim B., Bozk r A., Canefe K. Preparation and evaluation of surface-modified PLGA microparticles with chitosan for pulmonary delivery of proteins. 8th International Conference of The European Chitin Society, Antalya-TURKEY, September 8-11 (2007).

PO021

WGA functionalized chitosan-Ca-alginate microparticles loaded with 5-FU for local treatment of colon cancer

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INTRODUCTION

One approach in management of colon cancer might be a site-specific drug delivery using biodegradable polymeric DDS with muco/bioadhesive properties. The rational for the development of such systems for colon targeting is the presence of high level of polysaccharides of microbial origin in the human colon and the possibility of direct binding to the mucosal surface by nonspecific or specific interactions with surface ligands. Effective targeting to the colon might be obtained by nonspe-

cific interaction (passive accumulation) at the site of action using mucoadhesive polymers (natural polysaccharides) or by specific ligand-receptor interactions using biological molecules, such as lectins or antibodies. These interactions can result in improved targeting efficiency of the drug to the site of action *i.e.* active uptake of therapeutics in the target tissues or cells and better therapeutic efficacy.

We have designed a novel carrier system, polyanion/polycation hydrogel microparticles, loaded with 5-FU, with muco/bioadhesive and controlled release properties for local colon delivery. The delivery system consists of microparticulated acidoresistantly coated 5-FU incorporated within chitosan-Ca-alginate (CTS-Ca-ALG) matrix by one step spray-drying process [1, 2].

Having in mind that the main disadvantage of the mucoadhesive colloidal drug carrier systems is adherence to the substrate by non-specific interactions *i.e.,* polymers cannot distinguish between the adherence to intestinal mucus or to the surfaces of other gut parts or contents, prepared formulation was further functionalized by covalent linkage of WGA lectin to MP`s surface after activation of the surface –OH groups with CDI reagent.

EXPERIMENTAL METHODS

Preparation procedure

5-FU loaded CTS-Ca-ALG MP s were prepared by one step spray-drying process - PATENT No. MP/MK/05/01/FF/BE/01/IP (Buchi 290, Mini Spray Dryer, Swiss). The composition of the prepared formulations expressed as mass ratio was: CTS/ALG 0.5, $CaCl_2$/ALG 1.25 and 5-FU/total polymers 0.25.

Functionalized MP`s were prepared by WGA coupling onto MP`s surface using carbonyldiimidazole - CDI method. WGA conjugated MP`s were freeze-dried (- 40 °C, 0.75 mBa, period 24h).

Characterization of microparticles

Prepared formulations were characterized in terms of production yield and mean particle size (Malvern 2600, Multisizer, Malvern Instr., Ltd, UK). The particle size distribution was also expressed in terms of SPAN factor value.

The amount of bound lectin to the surface of the MP`s was determined by HPLC analysis.

The *in vitro* mucoadhesivity of the MP and MP-WGA was determined in different buffer systems (pH 2.0, 4.5, 6.8 and 7.4) using crude pig mucin (10% sialic acid content), UV spectrophotometrically at 258 nm. The PM binding efficiency of MP`s was calculated from the following equation: *PM binding efficiency (%) = (Co-Cs / Co) x 100;* where *Co* is the initial concentration of PM used for incubation and *Cs* is the concentration of free PM determined in the supernatant.

*Caco-2 cell culture studies:*To evaluate the potentiality of prepared formulations (MP, MP-WGA), as a systems for local colon delivery of 5-FU, for effective treatment of colon cancer, an *in vitro* studies were performed using a monolayers of Caco-2 cells.

Transport studies: 5-FU alone, MP and MP-WGA were dispersed in HBSS, pH 7.4 in final concentration of 0.1 mg/ml 5-FU. Transport studies were performed for 6 hours at 37 °C in shaking water bath. Samples were taken at appropriate time intervals, and assayed by HPLC. The *Papp* was also calculated.

Investigation of the in vitro *efficacy of MP`s loaded with 5-FU in Caco-2 cell cultures*: Caco-2 cells were incubated at 37 °C, for 30 min with studied formulations before the addition of [methyl-^{3}H] thymidine standard solution (100 Ci/ml). At 6th hour, the whole media was replaced with DMEM supplemented with 10% BSA. The incubation was continued to 24 hour. The radioactivity was counted with a liquid scintillation analyzer.

RESULTS AND DISCUSION

Production yield, mean particle size, SPAN factor and WGA conjugation efficacy of prepared formulations are presented in Table 1.

Table 1: Production yield, mean particle size, SPAN factor and WGA conjugation efficacy

	MP	MP-WGA
Production yield (% ± SD)	52.3 ± 1.9	36.29 ± 2.3
Mean particle size (μm ± SD)	6.64 ± 0.03	14.74 ± 0.05
SPAN factor ± SD	1.967 ± 0.05	2.28 ± 0.9
WGA conjugation efficacy (% ± SD)	/	50.44 ± 1.46

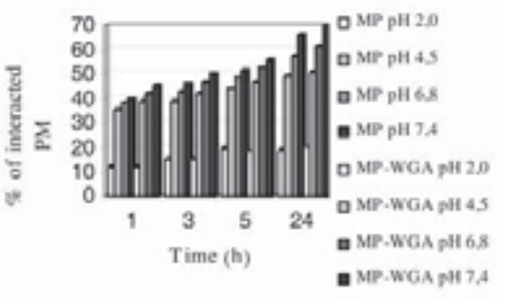

Figure 1: The effect of WGA conjugation on the amount of interacted PM with MP`s

Determined particle size of WGA conjugated MP`s (~15 μm) favors the formulation of dosage form with prolonged and controlled release properties for colon targeting and efficient treatment of colon cancer [3].

All prepared formulations showed excessive mucoadhesiveness due to the properties of chitosan-Ca-alginate matrix. Applying this optimized protocol on surface engineering of microparticles with WGA improved mucoadhesive characteristics of the beads was obtained (lectin/sugar recognition) (Fig.1).

In vitro studies using Caco-2 cell monolayer s demonstrated the feasibility of MP`s to affects the transport of 5-FU across the cell model depending of the formulation tested (5-FU sol. MP, MP-WGA), leading to

pronounced presence of 5-FU into the cells (Fig.2). WGA conjugated MP`s presented lowest *Papp* values.

Performed uptake studies (Fig. 3) suggested that immobilization of WGA onto surface of the particles, due to the improved MP/cell interaction, as well as the enhanced tissue accumulation of 5-FU, could led to improved efficacy in targeted anticancer colon therapy accompanied with lowering of toxic and side effects of the encapsulated drug substance.

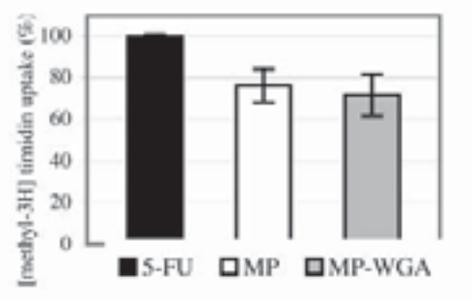

Figure 2: Transport studies through Caco-2 cell monolayers (n=20)

CONCLUSION

WGA functionalized MP`s, could be suitable candidates for controlled colon-specific delivery of 5-FU, opening a new therapeutic potential for this carriers for local treatment of colon cancer.

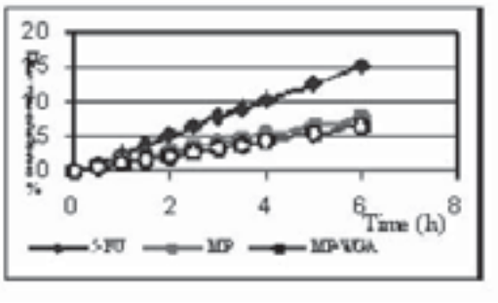

Figure 3: Effect of 5-FU incorporation in MP formulations in Caco-2 cells (n=10)

ACKNOWLEDGMENTS

Ministry of Education and Science of the R. Macedonia and TUBITAK, Turkey, grand No. 17-364 and NATO SfP: 978023.

References

1. Glavas-Dodov M., Calis S., Simonoska M., Goracinova K., Formulation and evaluation of 5-FU loaded chitosan/alginate microparticles prepared by spray-drying. 13th IPTS, 2006, Turkey.
2. Glavas Dodov M., Hincal A., Calis S., Simonoska Crcarevska M., Geskovski N., Goracinova K., Spray-dried chitosan-Ca-alginate microparticles for colon delivery of 5-FU, Maced. Pharm Bull, 53 (1, 2): 45-46 (2007)
3. Lamprecht A., Schafer U., Lehr C. M., Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa, Pharm Res, 18: 788-793 (2001)

PO022

The effectiveness of chitosan coating for prolonged 5-ASA release from calcium alginate microcapsules

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INTRODUCTION

Calcium–alginate coated chitosan (CACC) microcapsules have been extensively studied as drug delivery formulation. Most of the previous works has been oriented to the encapsulation of proteins. However, no work has been devoted to the study of the encapsulation of conventional low molecular weight drugs [1]. The aim of this study was to evaluate the effectiveness of chitosan coating for prolonged 5-ASA release from calcium alginate microcapsules.

EXPERIMENTAL METHODS

5-ASA microcapsules were prepared by adding 5-ASA (5 mg/ml) to 20 ml of aqueous solutions of sodium alginate (1.0% w/v). This dispersion was dropped into a 20 ml of gelling solution, containing $CaCl_2$ (1.0 %w/v) by using an hypodermic syringe. Microspheres formed immediately and were left in the original solution for 0.5 h. Then they were separated by sieving, washed three times with distilled water and dried in an oven at 50 °C for 12 h (5-ASA core, CA). The wetted CA microcapsules were submerged in 100 ml chitosan 1% w/v + $CaCl_2$ 1% w / v in acetic acid / sodium acetate 0.1 M buffer solution for 1h. Then they were treated the same way as describe above (CA coated with chitosan in two steps, CAC2).

Dissolution and swelling test: In a thermostated bath (Labtech, LWD-122D) were placed three tall 1L beaker equipped with a mechanical shaker (IKA, RW 20 DZM) with digital speed control, provided with paddle impeller. The dissolution studies were developed at 37 ± 0.1 °C and

50 ± 5 rpm. In each beaker were placed 900 ml of dissolution medium and 100 mg of microcapsules which were placed inside a basket of stainless steel mesh No. 40. Three dissolution media were used: HCl 0.1 M + M KCl 0.2 pH 1.2 (acid), distilled water pH 5.5 (water), and 0.05M KH_2PO_4 pH 7.5 (phosphate). Aliquots of 10 mL were taken at different sampling times and were replaced with an equal volume of the medium. The content of 5-ASA was measured by UV spectroscopy by using a UV-visible UNICAM UV3 spectrometer at 302 nm (acid), 298 nm (water), and 330 nm (phosphate).

The same equipment, dissolution media, and conditions describe above were used for swelling test. At each sampling time the basket was removed, the dissolution medium was eliminated and the basket was weighed. The degree of swelling (DS) was estimated as $DS=(M_t-M_0/M_0)*100$, where M_t=weight at time t and M_0= weight at time 0. All assays were done in triplicate.

RESULTS AND DISCUSSION

The mean dissolution time of CA and CAC2 were estimated from Weibull and Dobashi model for each dissolution medium. Figure 1 shows that the T_{50} of CAC2 is significantly lower in water and phosphate compared with CA. In both systems more prolonged drug release were obtained in the order acid<phosphate<water. These results iare in accordance with the disminished solubility of the 5-ASA (acid : 8.65 mg/ml; phosphate: 3.94 mg/ml; water: 0.84 mg/ml). The 5-ASA molecule has two pKa values (pKa_1= 2.3 and pKa_2= 5.4) with this values it is possible to estimate the different percentages of ionized species at different pH [2]. Thus, in acid medium the cationic species predominate while in water amphoteric and anionic species are present and in phosphate medium anionic species predominante. Alginate is fully ionized in water and phosphate but it is found in its unionized form in acid. Chitosan is ionised in acid and water but not in phosphate. These differences in the degree of ionization of chitosan and alginate and the ionized species for each dissolutiom media will affect the swelling behaviour of the microcapsules.

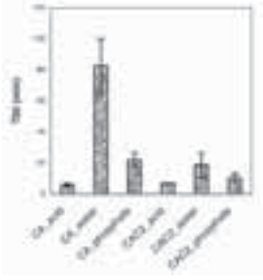

Figure 1: Mean dissolution time (T50) for CA and CAC2 in different dissolution media

Figure 2 shows the swelling behavior of CA and CAC2 in different dissolution media. In both systems there is a low degree of swelling in acid media. Then, the quick drug release is due to the high solubility of 5-ASA. For CA, there is a swelling-erosion process in phosphate but not in water, due to the solubility of calcium alginate matrix. This behavior explains the large difference in T_{50} obtained in water and phosphate. Instead for CAC2, there is no significant difference in T_{50} because in both media the swelling-erosion process occur.

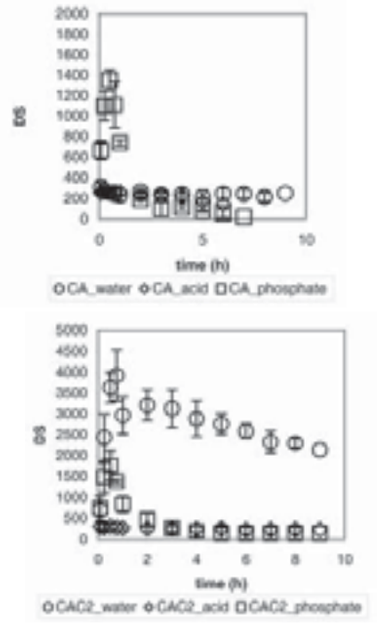

Figure 2: Swelling behavior of CA and CAC2 in different dissolution media

CONCLUSION

The chitosan coating over calcium alginate microcapsule was not effective to avoid the quick 5-ASA release in acid medium and increased the 5-ASA release in water and phosphate media.

ACKNOWLEDGMENTS

This work was supported by a grant from FONDECYT; Project no. 1061146

REFERENCES

1. Mladenovska et al. 5-ASA loaded chitosan–Ca–alginate microparticles: Preparation and physicochemical characterization. Int. J. Pharm. 345: 59-69 (2007).
2. Tapia et al. Study of dissolution behavior of matrices tablets based on alginate-gelatin mixtures as prolonged diltiazem hydrochloride release systems. Drug Dev. Ind.Pharm. 33: 1-9 (2007).

PO023

Prolonged release of bupivacaine and indomethacin from concentrated lipid microspheres

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INTRODUCTION

Lipids are promising candidates as components of biodegradable matrices incorporating drugs and the solid lipid nanospheres (SLN) with size below 0.5 m has been proposed as potential drug carriers [1]. Müller et al. [2] also recommended concentrated SLN or nanostructured lipid carriers (NLC) containing up to 80% of lipid phase as a new drug delivery system. Our research has been focused on solid lipid microspheres which are up to 30-50 μm in size and can be suitable for administration by other routes than intravenous (parenteral, ocular, rectal, oral) [3]. The process developed for lipid microspheres does not involve high pressure homogenization which is a critical step allowing particle sizes below 1 μm and is required for SLN.

The aim of the present study was to compare incorporation of bupivacaine base and indomethacin in concentrates of lipid microspheres (50% w/w of lipid) and to study the *in vitro* release rate of the incorporated drugs. The lipid cores were composed of a mixture of solid and liquid triglycerides (Precirol and Miglyol 4:1 or 9:1).

MATERIALS AND METHODS

• Materials

Bupivacaine free base was donated by Polfa Pharmaceutical Works (Warsaw, Poland) and indomethacin by Jelfa (Jelenia Góra, Poland). Precirol (glyceryl palmitostearate) was a gift from Gattefossé (Lyon, France). Miglyol 812 (medium chain triglycerides) was purchased from Caelo Caesar and Loretz (Hilden, Germany) and polysorbate (Tween 80) from Merck (Darmstadt, Germany).

• Concentrates of lipospheres

The content of lipids (Precirol in a mixture with Miglyol) in concentrates was 50% (w/w). In all formulations polysorbate 80 was used as an emulsifying agent (2.0% w/w) and glycerol was added for isotonicity (2.3% w/w). The formulations were prepared using a hot emulsification and cold resolidification method. Bupivacaine base or indomethacin was added into the melted lipid phase. The emulsification was performed at 80°C using a high-shear mixer Ultra-Turrax (IKA Labortechnik, Staufen, Germany) at 8000 rpm for 5 min. In formulations with bupivacaine pH was adjusted to 8.0 with NaOH. The bupivacaine content in the concentrates was 2% or 10% (w/w) and indomethacin – 1% w/w. The ratio of Precirol to Miglyol in the lipid cores was 4:1 (formulations 2B-2% and 2B-10% and I-1%) or 9:1 (1B-2% and 1B-10%).

• Analysis

Particle size distribution was measured using a laser diffractometer Mastersizer E (Malvern Instr., Malvern, UK).

The free drug content in the concentrates was evaluated by determination of drug concentration in the aqueous phase separated from the diluted formulations (1:1) by ultrafiltration in filtration units – Microcon YM-100 (cut-off 100 kDa, Millipore, Bedford, USA).

The drug release rate was studied using a dialysis bag method. The concentrate of lipospheres (1.0 g) was placed in the dialysis bag (m.w. cut 12400 Da, Sigma, St. Louis, USA) which was introduced to a beaker with 100 ml of phosphate buffer pH 7.4 at 37°C stirred with a magnetic stirrer. The acceptor fluid was sampled up to 48 or 96 h. For comparison the experiment was also performed for the indomethacin concentrate which was diluted 1:5 with water.

The concentration of the released drugs was analysed using HPLC methods.

RESULTS AND DISCUSSION

The size of lipospheres prepared with indomethacin and bupivacaine was 1-30 μm and 5-40 μm, respectively. Solubility of bupivacaine estimated semiquantitatively in bulk Precirol was 200 mg/g. Despite of such a good solubility in lipid the large expulsion of the drug was observed during preparation of the microspheres [4]. In the concentrates containing 2% or 10% of bupivacaine 82.5% and 28.9% of the total drug, respectively, was found in the aqueous phase. In the case of indomethacin 60.9% of the drug was found in the aqueous phase of the formulation. Fig. 1 presents the release profile of indomethacin from the concentrated lipospheres. Despite of the low incorporation into the lipid cores the drug is released slowly for 48 h. If the concentrate is diluted, however, the drug release is immediate.

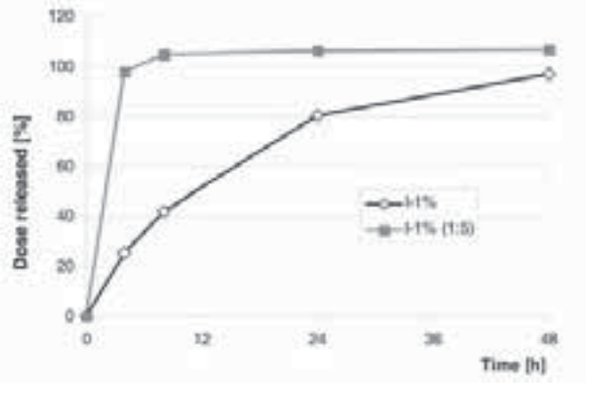

Figure 1: Release of indomethacin form the lipospheres concentrated and diluted 1:5 with water.

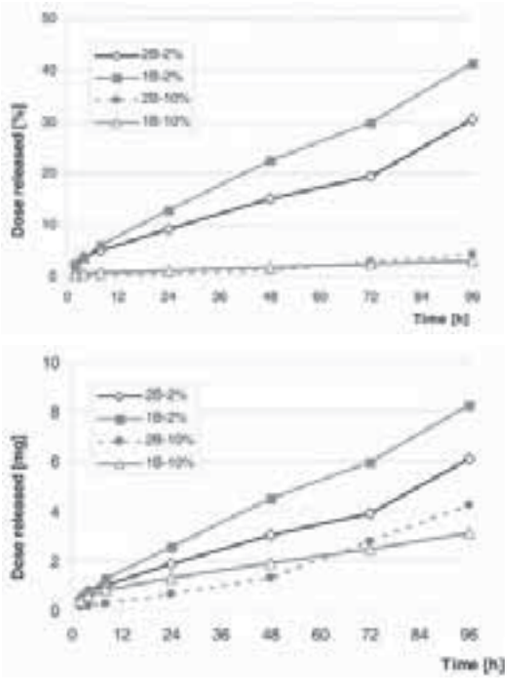

Figure 2: The effect of drug concentration (2% or 10%) and Precirol-Miglyol ratio (1B- 1:9 and 2B – 1:4) on the release of bupivacaine (% of the total dose and mg) from the concentrates of lipospheres.

The prolonged release of bupivacaine is even more evident, as shown in Fig. 2. Despite of the low incorporation of bupivacaine to the cores of lipid microspheres, the drug is very slowly released, with the rate depending on the drug concentration. Slower release of the drug from the concentrates containing 10% w/w of bupivacaine is related to the thicker consistency of the concentrate. On the other hand only small difference was observed when the structure of the lipids building the lipospheres was modified by adding 10% or 20% of the liquid lipid (Miglyol in 1B and 2B formulations).

CONCLUSION

Despite of the fact that the drug is not incorporated into the lipid cores of the lipospheres the prolonged release of the drugs was demonstrated for concentrated lipid microspheres (50% w/w of lipid).

ACKNOWLEDGEMENTS

The authors wish to thank Gattefossé for donating Precirol.

References

1. Müller R.H. et. al. Eur. J. Pharm. Biopharm. 50, 161-177 (2000)
2. Müller R.H. et al... Int. J. Pharm. 242, 121-128 (2002)
3. Pietkiewicz, J., Sznitowska, M. Pharmazie 59, 325-326 (2004)
4. Pietkiewicz J. et al. Int. J. Pharm. 310, 64-71 (2006)

PO024

Chitosan-tartrate microparticles for sustained release of vancomycin hydrochloride

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INTRODUCTION

Chitosan is a natural cationic biopolymer produced from N-deacetylation of chitin, a ß-(1-4)-linked N-acetyl-D-glycan. Chitosan's unique characteristics make it potentially useful in a variety of applications, such as a drug carrier, wound healing, implantation and gene therapy because of the following advantages: chitosan is inexpensive, non-toxic, biodegradable and biocompatible. Vancomycin hydrochloride (VH) was used as a model peptide drug. Following oral administration, peptide drug suffer from poor intestinal absorption due to the susceptibility of these drugs to the proteolytic enzymes in the gastro-intestinal tract and poor membrane permeability. The absorption of peptide drugs could be enhanced in the colon because of the low activity of proteolytic enzymes there and the long residence time. In this study, chitosan microparticles were prepared using a spray-drying technique. Scanning electron microscopy and Fourier transform infrared spectroscopy were carried out to understand respectively the morphological analysis and the structural interactions between chitosan and tartaric acid. The release of VH was also estimated.

EXPERIMENTAL METHODS

Microparticles preparation

0.25 g chitosan (1.55 mmoles glucosamine) were dissolved in 50 ml water containing tartaric acid in molar ratios: 1:5 moles monomer:moles acid (1:5 CH monomer/Tar) at room temperature. The solution was spray-dried (Buchi, Mini Spray Dried, B-121, Switzerland). The drying conditions were as follows: inlet temperature 105 °C; outlet temperature 105 °C; air flow rate 600 Nl/h and flow rate 50 l/h.

Microparticles characterization

Infrared (IR) spectra were recorded with a Jasco FT-IR-410 spectrophotometer. The samples were prepared by processing compressed KBr disks.

The morphology of microparticles was analysed by scanning electron microscopy (SEM). The microparticles were fixed on supports and coated with gold-palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with LEO 420 (LEO Electron Microscopy Ltd., Cambridge, England).

Preparation of the spray-dried drug-polymer physical mixture

1.55 mmoles of chitosan tartrate (0.46 g of 1:5 CH monomer/Tar) and 1.55 mmoles vancomycin hydrochloride (2.30 g) were weighed and mixed in a mortar until homogeneity.

***In vitro* release studies**

To detect the amount of free drug available from the drug-polymer mixture, the solid mixture (50 mg) was introduced in a donor cell containing 3 ml of pH 2.0 separated by a dialysis membrane (MW cut off = 14000) from a receiving compartment containing 10 ml of the same aqueous buffer, which was replaced after time intervals suitable to guarantee sink conditions throughout the runs. In order to simulate gastrointestinal conditions, the donor and receiving compartment pH was maintained at pH 2.0 for 3 h, at pH 5.5 for 2 h and at pH 7.6 up to 48 h. The system was thermostated at 37 °C and both compartments (donor and receiving) were stirred. The drug was spectrophotometrically analyzed in the receiving phase.

RESULTS AND DISCUSSION

FTIR

CH showed the characteristic band of the amino group at 1669 cm^{-1}. For CH- Tar salt, the band of amino group at 1669 cm^{-1}, which can be observed clearly in pure chitosan, decrease dramatically and two new peaks at 1716 and 1741 cm^{-1}, which can be assigned to the absorption peaks of the carboxyl groups of tartaric acid (the absorption peak of carboxyl groups in pure tartaric acid appears at 1740 cm^{-1}), and the NH_3^+ absorption of CH, respectively, are observed. It was confirmed that tartaric acid might interact with chitosan at the position of an amino group to form chitosan salt (Figure 1).

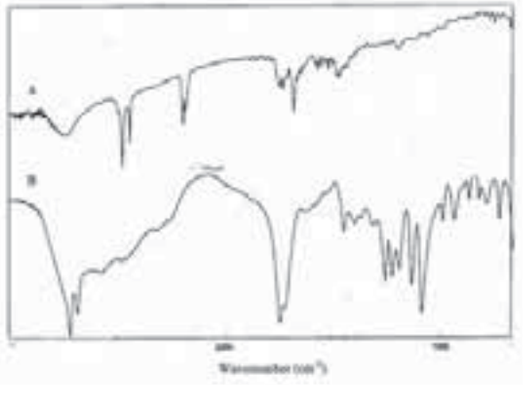

Figure 1: FT-IR spectra of A) CH, B) CH-Tar.

SEM

The SEM of spray-dried chitosan tartrate microparticles appeared to have a irregular size with a high tendency to aggregation (Fig. 2A). In the case of physical mixtures, liophilized drug is evident surrounded by chitosan tartrate (Fig. 2B).

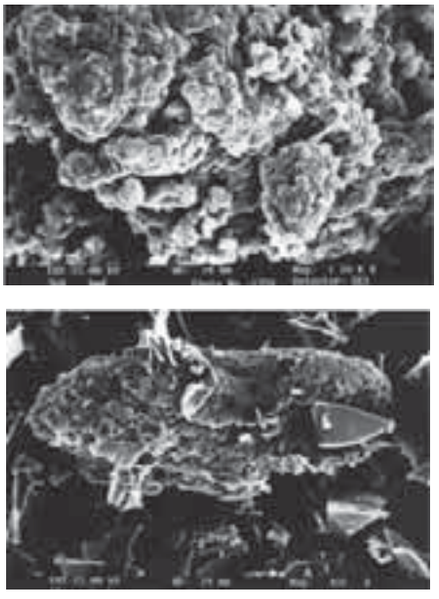

Figure 2: SEM of (a) chitosan tartrate microparticles and (b) physical mixture of chitosan-tartrate and VH.

In vitro release studies

The release from physical mixtures was studied in solutions at pH 2.0, 5.5 and pH 7.6, which simulated gastric and intestinal environments respectively. Drug availability, expressed as fractional release over time, was lower from physical mixtures than the pure drug at each pH analysed. No release was seen in acidic pH for 3 h, but at higher pH swelling of chitosan-tartrate started leading to sustained release drug (Fig. 3).

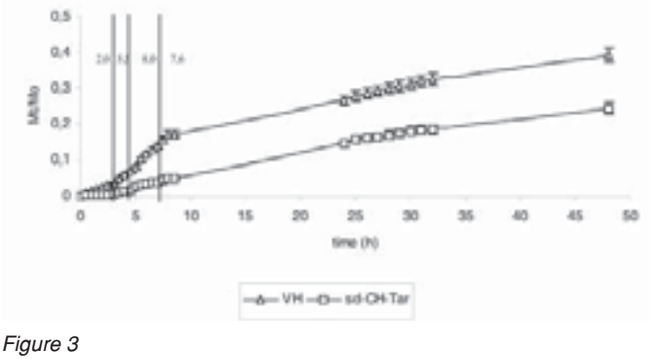

Figure 3

CONCLUSION

The CH-tart can be prepared by spray-drying method. In vitro release studies indicate that the physical mixture of the chitosan tartrate with VH can be used as vehicle for the administration of hydrophilic drugs.

PO025

Spray-dried chitosan microspheres with lecithin nanocores as cyclosporin A delivery system

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INTRODUCTION

Cyclosporin is a highly lipophilic neutral cyclic peptide consisting of 11 amino acids. Its low water solubility present a serious problem causing undesirable biopharmaceutical properties, such as erratic bioavailability from oral and topical routes (1). To overcome this problem bioadhesive drug delivery system like chitosan microspheres could be used (2). Chitosan, a polycationic biopolymer is well known for its several favorable biological properties such as biodegradability, nontoxicity and biocompatibility (2). In this study spray-drying method was used for preparing chitosan microspheres with lecithin nanocores as delivery system for cyclosporin A (CyA). The swelling properties of the microspheres and in vitro CyA release were investigated.

MATERIALS AND METHODS

Chitosan (Protasan UP Base, NovaMatrix, Norway); cyclosporine A (Galena, Czech Republic); soybean lecithins, Lipoid S100 and S45 (a kind gift from Liopid GmbH, Germany). Simulated Tear Fluid (STF), was prepared as an aqueous solution containing 6.7 g NaCl, 2.0 g $NaHCO_3$ and 0.08 g $CaCl_2 \cdot 2H_2O$ per litre.

Chitosan base (CB) and chitosan hydrochloride (CS) were dissolved at 2 % (w/v) concentration in 0,5 % (v/v) acetic acid solution and in distilled water, respectively.

Microspheres were prepared as follows: lecithin (L) (4 mg/ml) and CyA (1,27 mg/ml) were disolved in absolute etanol and 22,5 ml of such solution injected into 35 ml of distilled water under magnetic stirring (900 rpm). CB solution or a mixture of CB and CS solutions (3:2, v/v) was then added to L/CyA dispersion, obtaining CyA/chitosan ratio of 10:90 (w/w). Dispersion was subjected to spray-drying (Buchi 190 mini spray dryer, Switzerland). The spraying conditions were as follows: sample and air flow rate of 0,25 l/h and 700 Nl/h, respectively, inlet and outlet temperature of 150°C and 80°C, respectively.

For determination of the CyA loading efficiency, the microspheres were suspended in absolute ethanol (1,2 mg/ml) and shaken for 24 h at 300 rpm., than sonicated for 2 h. The suspensions where than centrifugated at 3000 g for 30 min and the supernatant was filtered and evaporated to dryness. The residue was dissolwed in 30 ml solution of acetonitrile and water (1:1, v/v) and the CyA content was determined by HPLC analysis at 210 nm.

The water-absorbing capacity of each microsphere sample was determined by a volumetric method using a Franz diffusion cell apparatus. A water-permeable polyamide membrane with 0.45 μm pore size was placed between the microsphere sample (3 mg) and receptor cell, which was filled with STF. The level of STF in graduated part of Franz diffusion cell lowered due to liquid uptake of the microspheres. The amount of STF, equal to the amount of STF absorbed by the microspheres, was then added to the receptor cell. The liquid uptake of each sample was expressed as a volume of STF added per milligram of the microspheres in 15 min swelling process.

In vitro drug release was performed in 20 ml STF pH 6,8 at 37 °C and 50 rpm. At fixed time intervals 0,5 ml samples were withdrawn and replaced with the same volume of dissolution medium. CyA content in the dissolution samples was measured by HPLC analysis at 210 nm. The dissolved amount of drug at each time was expressed as a percentage of the dose. Each experiment was performed in triplicate.

RESULTS AND DISCUSSION

The microsphere samples and their composition are presented in Table 1. The spray-drying method used resulted with microspheres (mean diameter size 2-3 μm) of good morphological characteristics and relatively high production yields (46-54 %).

Table 1: Composition and main characteristics of spray-dried CyA-loaded microspheres

Sample*	Production yield (%)	Drug loading (%)	EE (%)*	STF volume (μL/mg)*
CB	53	7,2	73	-
L100/CB	49	6,5	88	53
L100/CB/CS	54	7,0	91	23
L45/CB/CS	46	6,5	86	20

* CB = chitosan base; CS = chitosan hydrochloride;
L100 = lecythin S100; L45 = lecythin S45,
EE = entrapment efficiency; STF = simulated tear fluid

The entrapment efficiencies were always very high (Table 1). Due to the lipophilic nature of lecithin, spray-drying of lecithin/chitosan systems resulted in improved CyA entrapment (86-91 %) when compared to simple chitosan solution (73 %; sample CB). However, neither the type of

lecithin (S100 or S45) nor chitosan type (CB or CB/CS) used for the microsphere preparation influenced on the CyA entrapment.

CyA-loaded microspheres swelling capacity was expressed as absorbed volume of STF per milligram of the microspheres (Table 1). CB microspheres showed no swelling ability, while L/CB and L/CB/CS microspheres absorbed STF volume proportional to CB content in the preparation. It can be assumed that CB microspheres, despite the highest content of CB in the preparation, showed no swelling ability due to the presence of lipophilic CyA at the microspheres surface, thus influencing its characteristics. Higher swelling abilities of lecithin/chitosan microspheres can be ascribed to lecithin inducement and consequent better CyA entrapment, resulting in more hydrophilic nature of the microspheres prepared.

The release profiles of CyA from the microsphere prepared are shown in Fig.1. Comparing L100/CB and L100/CB/CS microspheres, CyA release from the microspheres increased with CB content and swelling ability of the microspheres, suggesting that matrix swelling improved CyA solubility. However, CyA release profile from CB microspheres was similar to release profile from L100/CB microspheres, despite the great difference in their swelling properties. The fast CyA release from CB microspheres is in agreement with the previous assumption that CyA is present at the CB microspheres surface. Comparing L100/CB/CS and L45/CB/CS microspheres, it may be concluded that the type of lecithin influenced the CyA release profile.

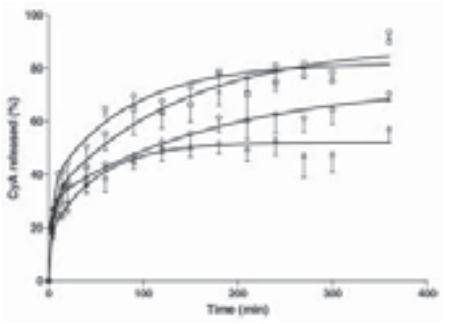

Figure 1: Release profiles of CyA from CB (○), L100/CB (□), L100/CB/CS (△) and L45/CB/CS (◇) microspheres in STF pH 7,4 at 37 °C and 50 rpm. Values are mean ±SD (n=3).

CONCLUSIONS

A new method for the preparation of spray-dried CyA-loaded hydrophilic microspheres was developed. Incorporation of lecithin nanocores into hydrophylic chitosan matrix resulted in improved CyA entrapment, swelling ability and control over CyA release.

References

1. Ludwig, A., The use of mucoadhesive polymers in ocular drug delivery, Adv. Drug Deliv. Rev. 57: 1595-1639, 2005.
2. Italia, JL., Bhardwa, V., Kumar, MNVR, Disease, destination, dose and delivery aspects of ciclosporin: the state of the art, DDT, 17/18: 846-854, 2006.

PO026

Influence of polyethylene glycol on the properties of poly(malate) and poly(lactide-co-glycolide) microparticles

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INTRODUCTION

Biodegradable drug delivery systems have been of particular interest because of possibility either to sustain or to regulate drug release process. In addition, an including of labile drug molecules into polymeric particles could improve their stability and further, better efficiency of the therapy could be expected. The copolymers of poly(lactic-co-glycolic acid) are widely applied as carriers for micro- and nanoparticles. The copolymers of malic acid are less investigated although they are also biodegradable and biocompatible. Recently, microparticles were developed on the base of malic acid polymers by polycondensation method.

The aim of the present study was to investigate the possibility to modify the properties of the poly(malate) microparticles by incorporation of low molecular polyethylene glycol (PEG 400). In parallel, microparticles based on PLGA-copolymer were also developed and modified with PEG 400.

METHODS

Preparation of cross-linked poly(malate) microparticles

The cross-linked poly(malate) microparticles were prepared by polycondensation method. Thus, 1.0 mmol dipyridamole (0.505g), 1.25 mmol pyridine (0.17ml) and 11.0mmol dicyclohexylcarbodiimide (2.28g) were dissolved in tetrahydrofuran (THF). The organic phase was added dropwise to a solution of 5.0 mmol D,L-malic acid (0.67g) and 0.4 mmol p-toluenesulfonic acid (0.08g) in THF. The reaction was carried out at 40-45 °C temperature and continued for 24 hours. Then, the mixture was filtered and the yellow precipitate of the cross-linked poly(malate) microparticles (PMAL-MP) was subsequently purified by washing with hot ethanol. Finally, the resulted microparticles were rinsed with diethyl ether and dried. The modified poly(malate) microparticles (PMAL-PEG-MP) were obtained applying the same protocol but with addition of PEG 400 (0.335g) into the organic solution of the drug.

Preparation of PLGA-microparticles

The weighed amount of PLGA (0.9g) and dipyridamole (0.1g) were dissolved in 15 ml mixed phase containing chloroform:acetone (1:1, v/v). The organic solution was subsequently added to 150 ml of aqueous phase containing PVA (1% w/v) under stirring at 800 rpm and the emulsification was carried out during 30 min. The resulted suspension was poured into 500 ml distilled water under stirring at 600 rpm. After one hour, the solidified microparticles were collected by centrifugation (10 000 rpm, 10 min.), washed with distilled water and dried under vacuum at room temperature for 24 hours. The modified PLGA-PEG-MP were prepared following the same procedure but PEG 400 was added to the organic phase in the concentration of 10 mg/ml.

Drug loading

The microparticles were hydrolyzed in 1N NaOH medium and after suitable dilution the absorbance of samples was measured spectrophotometrically (λ=284 nm). The amount of dipyridamole was determined using standard plot prepared in the concentration range of 4-20 μg/ml (r=0.999).

In vitro drug release

In vitro drug release was conducted in a shaker bath (75 rpm) at 37°C temperature in a phosphate buffer (pH=7.4). The amount of the released dipyridamole was determined by HPLC method (UV-detector Waters 991 and a column Bondapak C_{18}). Determinations were made at a flow rate of 0.4 ml/min using methanol:water (30:70 v/v) as a mobile phase and UV-detection adjusted at 284 nm.

Preparation and characterization of poly(malate) and PLGA-microparticles

The availability of free functional groups in the compounds (malic acid and drug molecule) is the main prerequisite for preparation of cross-linked poly(malate) microparticles via polycondensation. The participation of PEG into cross-linking was expected taking in account the availability of hydroxyl functional groups. Scanning electron microscopy revealed that the size of the resulted PMAL-PEG-MP was similar to that of non-modified PMAL-MP, ranging from 1 to 15 μm. The microparticles prepared on the base of PLGA (PLGA-MP and PLGA-PEG-MP) were also spherical, but slightly larger (5-50 μm). Drug loading of these microparticles was lower than the loading achieved in both types of poly(malate) microparticles (Table 1). This fact was probably associated with the different affinity of the model drug to the both polymeric carriers as well as the method of preparation. On the other hand, it seemed that the addition of PEG 400 reduced dipyridamole loading into the poly(malate) particles. The reason for the lower drug loading of PMAL-PEG-MP could be the competition between hydroxyl groups of PEG and dipyridamole during cross-linking.

Table 1: Drug loading and encapsulation efficiency obtained for the poly(malate) and poly(lactic-co-glycolide) microparticles.

Sample	Drug loading (%)	EE (%)
PLGA-MP	4.3 ± 0.7	43.2 ± 6.8
PLGA-PEG-MP	3.9 ± 0.8	47.0 ± 9.7
PMAL-MP	30.4 ± 1.4	62.6 ± 2.8
PMAL-PEG-MP	13.5 ± 2.3	49.1 ± 8.6

In vitro drug release from poly(malate) and poly(lactide-co-glycolide) microparticles

The drug release from the cross-linked poly(malate) microparticles was characterized with two phases – lag-time phase and sustained release phase (Fig. 1). Both phases were due to the cross-linking density of the poly(malate) system and the cleavage of the bonds between poly(malic acid) fragments and drug. The release profile of PMAL-PEG-MP differed than the profile of the non-modified PMAL-MP. The complete release of dipyridamole from PMAL-PEG-MP was achieved for 22 days, while the release process from PMAL-MP took 38 days. The reason for the faster release from PMAL-PEG-MP would be the hydrophilic properties of PEG and the formation of porous structure of the cross-linked particles.

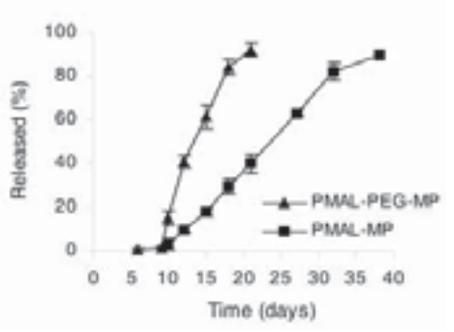

Figure 1: In vitro release profiles of dipyridamole from poly(malate) microparticles (pH 7.4).

Drug release from the poly(lactide-co-glycolide) particles occurred in a biphasic manner – initial burst phase (slightly higher for PLGA-PEG-MP) and sustained release phase (Fig. 2). Complete release from PLGA-PEG-MP occurred for 26 days, while at the same time 84% dipyridamole was released from PLGA-MP. This fact could be attributed to the enhanced degradation of the polymeric matrix as a consequence of the high affinity of PEG to the release medium.

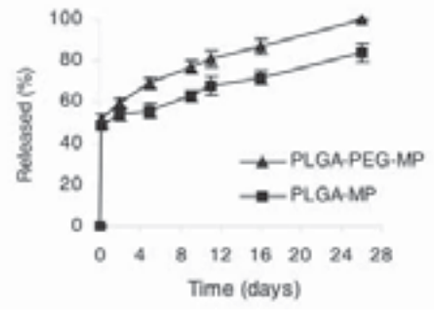

Figure 2: In vitro release profiles of dipyridamole from poly(lactide-co-glycolide) microparticles (pH 7.4).

CONCLUSION

The modification of both types of microparticles with low molecular polyethylene glycol could be used aiming to achieve appropriate release of antithrombotic drug like dipyridamole.

PO027

Phenytoin-loaded microparticles of alginate and alginate chitosan: preparation and characterization

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INTRODUCTION

Alginate-based microparticles have been widely investigated for a large array of applications like enzyme immobilization, immunoisolation in cell transplantation and drug release systems. Concerning the sustained release systems, gel erosion of alginate-based microparticles due to presence of chelators such as phosphate, lactate or citrate or non-gelling cations like sodium or magnesium, imposes a significant problem since it accelerates release of drug. Therefore, there is a need for a stabilizing agent, which can be a membrane forming polycation bound to the alginate gel by electrostatic interactions. One such agent is chitosan, a polycationic polysaccharide derived from natural polymer chitin [1].

In the present study we performed two different methods of alginate based microparticles preparation: with and without treatment with chitosan. The model substance phenytoin has been well established as a standard antiepileptic agent. The extended-release formulations of antiepileptics decrease daily fluctuations and simplify treatment of this chronic condition. Such commercial formulations with phenytoin sodium appear to be well designed, with one possible shortcoming. That concern deals with the potential for irregular absorption that appears to occur particularly in elderly patients. The final aim of the project was to investigate microparticles loaded with phenytoin in its acidic form, with possibly improved liberation profile [2]. The additional benefit might be manufacture in environmentally friendly conditions.

EXPERIMENTAL METHODS

Materials

Sodium alginate with high guluronic acid ("high G") content was kindly donated by ISP (International Specialty Products, New Jersey, USA). Phenytoin was provided by Sigma-Aldrich (St. Louis, USA). Chitosan, high molecular weight, was purchased from Aldrich (Milwaukee, USA).

Methods

Microparticles preparation

Microparticles were prepared using a custom made air-jet device with, approximately, 7ml of 2% suspension of phenytoin in 2% aqueous solution of alginate (drug: polymer ratio = 1:1) extruded through a 20 gauge needle at a rate of 120 ml/h into 70 ml of a gelling medium consisted of aqueous solution of different concentration of calcium chloride, chitosan and acetate buffer. The laminar air flow in coaxial cylinder, pointed to the tip of the needle producing small droplets, was constant (1300 ml/h). The prepared microparticles were collected, washed and dried at room temperature for 72h. Four different formulations (F1-F4) were made (**Table 1).**

Table 1: Composition of samples

Formulation	$CaCl_2$ concentration (%, w/w)	Chitosan concentration (%, w/w)
F1	0.5	-
F2	2.0	-
F3	0.5	0.2
F4	2.0	0.2

Surface morphology and particle size analysis

The surface morphology was determined by scanning electron microscopy (SEM) DSM 940 A (Zeiss, Oberkochen, Germany) using gold sputter technique. The particle sizes of 50 microparticles were measured with Motic digital microscope DMB3-223ASC for each formulation and the mean particle size was determined.

Encapsulation efficiency determination

Determination of phenytoin in microparticles was performed using RP-HPLC method, with phenobarbitone as an internal standard. The column used was Zorbax SB-C18, 250x4.6 mm, 5 μm.

Drug release studies

The release of phenytoin from microparticles was measured quantitatively using the rotating basket method at 100 rpm (37°C), in 900 ml phosphate buffer (pH 7.4 USP23).

Differential scanning calorimetry (DSC)

DSC thermograms were obtained using a differential scanning calorimeter STA 409 PG (Netsch, Selb, Germany). Samples were crimped in a standard aluminum pan and run at a scanning rate of 20°C/min from 25 to 330°C.

RESULTS AND DISCUSSION

Recorded microparticle sizes were in range of 466.2 14.8 to 565.5 6.2 micrometers. The lack of distinct differences points to reproducibility of the chosen manufacturing parameters, with no influence of the chitosan addition on the mean microparticle size. Topographical SEM analysis revealed the presence of marked fractures and the particles of the free

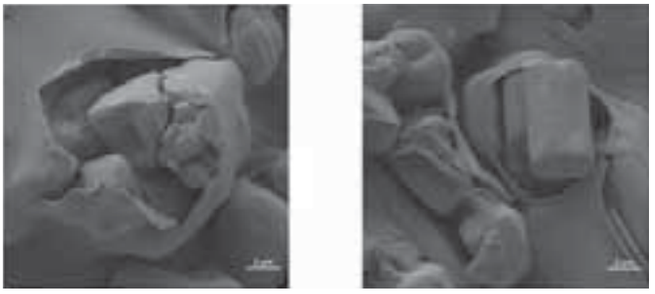

Figure 1: SEM micrographs and surface morphology of samples F1 (left) and F2 (right).

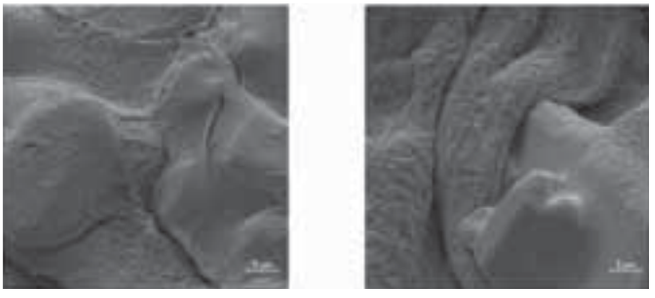

Figure 2: SEM micrographs and surface morphology of samples F3 (left) and F4 (right).

drug on surface of chitosan non-treated microparticles only (Fig. 1 and Fig 2). Furthermore, these morphological alterations did not affect encapsulation efficiency. All studied formulations were of high model drug loading (91-96%), with no influence of calcium chloride or chitosan concentration on this parameter. Increasing calcium concentration resulted in enhanced phenytoin release. The release of phenytoin held within the chitosan treated microparticles incubated in phosphate buffer was delayed compared to the non treated ones, presumably due to the suppressed erosion of chitosan reinforced calcium-induced alginate gel

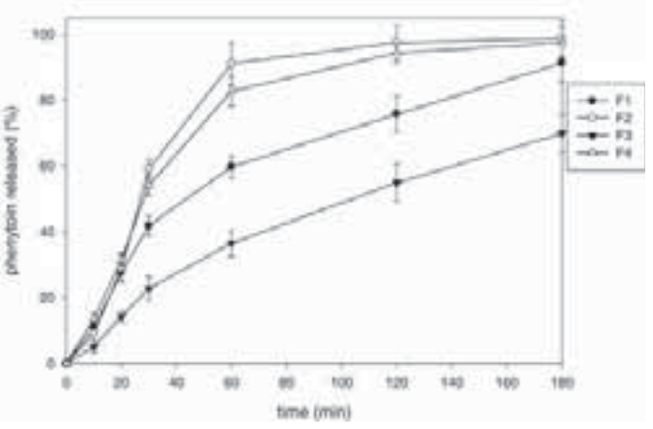

Figure 3: Release profiles of the studied samples.

network. The delay was distinct in the case of formulation F3, with the release half-time of 115 min (Fig. 3). DSC analysis demonstrated calcium-alginate and alginate-chitosan interaction, as well as absence of any interaction of the model drug, phenytoin (thermograms not presented).

CONCLUSION

The present study confirms the applicability of the tested manufacturing procedures of microparticles for incorporation of poorly soluble drugs, such as phenytoin.

References

1. Gaserod O, Sannes A, Skjak-Braek G. Microcapsules of Alginate-Chitosan. II. A Study of Capsule Stability and Permeability. Biomaterals 20:773-783 (1999).
2. Pellock JM, Smith MC, Cloyd JC, Uthman B, Wilder BJ. Extended-Release Formulations: Simplifying Strategies in the Management of Antiepileptic Drug Therapy. Epilepsy & Behavior 5:301-307 (2004).

PO028

Formulation of Meloxicam microparticles for dry powder inhalation

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INTRODUCTION

Modern pharmaceutical technology is concentrated on new drug forms, which are targeted to the exact site at the appropriate time, with maximum efficiency and reduced side-effects. Enhancement of the solubility of poorly-soluble drugs is one of the most important tasks in this field [1]. During recent decades, the lungs have been considered a promising route for the administration of drugs, not only for local treatment, but also for systemic therapy. There are several advantages in delivering drugs to the lungs: a large surface area for absorption (75 m^2), the abundance of capillaries which allow rapid absorption, and the thinness of the air-blood barrier. Dry powder inhalers (DPIs) are common delivery vehicles for pharmaceutical aerosols, with enhanced storage stability and ease of use for the patient. The performance of DPI systems is strongly dependent on formulation factors, and also on the construction of the delivery device and the patient s inhalation technique [2]. Most dry powder formulations consist of a micronized drug in an interactive mixture with lactose or mannitol as carrier, to which the drug is bound by adhesive forces. The particle size of the drug is a major factor in DPI formulations, with an accepted optimum size between 0.5 and 7 μm [3].

The aim of this work was primarily to produce and characterize particles of respirable size containing Meloxicam (MEL) and additives, by using wet milling combined with a spray-drying process. MEL is a nonsteroid anti-inflammatory drug that belongs in the enolic acid class of oxicam derivatives. It is a selective cyclooxygenase (COX-2) inhibitor used to treat rheumatoid arthritis, osteoarthritis and other joint diseases. MEL has an antifibrotic effect when used in a COX-2 inhibition approach as an adjuvant for the abatement of pneumopathy, and mixed with antibiotics it is useful in pneumonia therapy [4]. According to the biopharmaceutical classification system, MEL is a Class II drug, since it is poorly water-soluble and well permeable. Accordingly, our second aim was to improve its solubility and dissolution rate during the micronization process.

EXPERIMENTAL METHODS

• Materials

Meloxicam (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-benzothiazine-3-carboxamide-1,1-dioxide) was from EGIS Ltd. (Budapest, Hungary). Polyvinylpyrrolidone (PVP) K-25 was from BASF (Germany), and Tween 80 (polysorbate 80) and mannitol were from Hungaropharma Rt.

• Preparation of samples

Micronized products (MPs) were prepared from 1 g each of MEL and mannitol, 0.1 g of Tween 80 (T) and/or 0.05 g of PVP K-25 (P) ad 20 g of water, using an Ultraturrax (UT) (T-25 IKA-WERKE Germany) at 6500 and 24000 rpm and a Büchi Mini Dryer B-191 (Switzerland).

Table 1: Compositions of samples

Samples	MEL (g)	Mannitol (g)	Tween 80 (g)	PVP K-25 (g)
MP	1	1	-	-
MP-T	1	1	0.1	-
MP-P	1	1		0.05
MP-T+P	1	1	0.1	0.05

A physical mixture (PM) was prepared from MEL and mannitol in a 1:1 mass ratio, as a control.

• Investigations

Physico-chemical characterizations were performed by determination of the contact angles (Dataphysics OCA 20, Dataphysics Inc., GmbH, Germany), solubility tests at 362 nm (Perkin Elmer – Lambda 20 spectrophotometer, Germany) and particle size distribution (LEICA Q500MC, LEICA Cambridge Ltd., England, and Malvern Instruments, Worcestershire, UK). Morphological investigations were carried out by scanning electronmicroscopy (Hitachi S4700). *In vitro investigations* involved dissolution tests (USP rotating-basket dissolution apparatus, type DT) with kinectics in simulated media. *Structural evaluations* were performed by microscopic observations (LEICA MZ 6, Germany) and DSC measurements (Mettler Inc., Schwerzenbach, Switzerland).

RESULTS AND DISCUSSION

The *particle size analysis* revealed that the sizes of the MEL particles were decreased significantly, into the inhalable range (Table 2).

The *wettability study* indicated that the products had a hydrophilic character as compared with MEL (~70°). Significantly lower contact angles were measured for all samples, the decrease ranging from 52° to 22°. The *SEM images* of the raw MEL and the spray-dried powders demonstrated that the powder consisted of regular, spherical microparticles.

Table 2: Particle sizes of MEL and products after spray-drying

Sample	Particle size of MEL (m) SD
MEL	85.39 6.69
PM	83.26 4.12
MP	1.21 0.41
MP-T	4.58 0.36
MP-P	2.50 0.28
MP-T+P	2.44 0.07

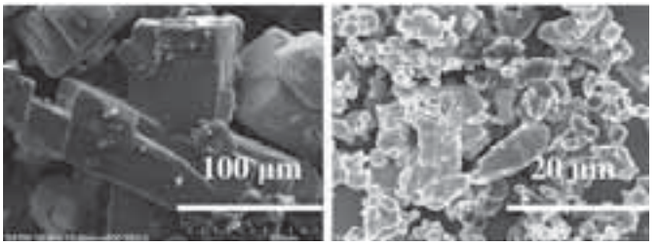

Figure 1: SEM images of MEL and MP-P samples

During the *dissolution tests,* the PM exhibited the same profile as MEL; only a 10% increase was detected after 60 min relative to the raw material. The MPs containing additives gave close to 100% release in the first 10 min. The dissolution rate in the first 5 min was ~30 times higher than for MEL and PM (Fig. 2).

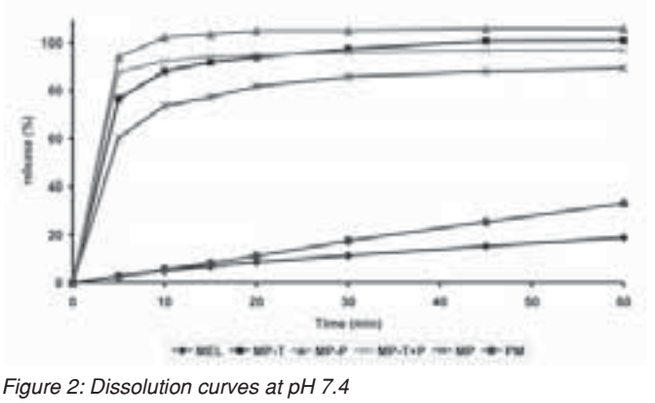

Figure 2: Dissolution curves at pH 7.4

The *DSC analysis* indicated that the samples were in crystalline form (stable form), but the spray-drying process often resulted in amorphous materials. The DSC curves of the starting compounds exhibited a sharp endothermic peak at 165 °C, corresponding to the melting point of mannitol, and a peak at 257 °C, the melting point of MEL. The melting point of MEL is followed by an exothermic peak, which refleczs the transformation or recrystallization of the drug material. For the samples, this melting was found around 230 °C. The *thermomicroscopic* pictures revealed that the MEL particles were melted in the melted mannitol.

CONCLUSIONS

The goal of this study was to produce a fine powder of spherical particles with a size appropriate for pulmonary delivery, as the therapeutic effect of MEL in the lung has been reported in the literature. Our study has led to a technological formulation through the use of wet milling which yields 2-5 μm particles with 100% drug release.

References

1. Ambrus, R. Aigner, Z., Soica, C., Peev, C. and Szabó-Révész, P. *Rev. Chim.* (2007) Vol. 58 No. 2 206–209.
2. Telko, M. J., Hickey, A. J. *Respiratory Care* (2005) Vol. 50. No. 9 1209-1227.
3. Davies, P. J., Hanlon, G. W., Molyneux, A. J. *J. Pharm. Pharmacol.* (1976) Vol. 28. 908-911.
4. Arafa H. M. M. et al. *Eur. J. Pharmacol.* (2007) Vol. 564. 181-189.

PO029

Preparation and evaluation of immediate release microparticles using hot-melt fluid bed technique

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INTRODUCTION

Hot-melt methods employ molten or softened materials (polymers, waxes, lipid-based materials) to act as binders in the preparation of solid dosage forms such as tablets, microparticles, pellets and granules or use such materials for coating of pharmaceutical formulations. The methods allow the preparation of either immediate release dosage forms or controlled release dosage forms, dependent on the materials employed. The most important advantage of these methods is the absence of solvents in the formulation preparation. This avoids issues of residual solvents, allows the processing of water sensitive drugs and reduces costs and time of production since there is no need for drying of the product. The application of the fluid bed system for preparation of formulations based on hot-melt technology was first described in 1990 by Jozwiakowski *et al* [1]. To date, fluid bed technology has been used to prepare agglomerates using polyethylene glycol, poloxamer 188, Gelucire 50/13 or glyceryl monostearate as meltable binders. Processes have been studied in terms of mechanism of agglomerate growth and the influence of binder droplet size, viscosity, starting material size and type of binder used. Fluid bed systems have also been used for hot-melt coating, where melts were applied as coatings to ensure prolonged release of active substances [2].

In our work we prepared microparticles using fluid bed rotor technology. We used different materials as binders to prepare particles with immediate release profile. Prepared microparticles were characterized in terms of dissolution rate of clarithromycin. In our conclusion, we discuss the influence of process parameters and note the possible optimizations or limitations of binders employed.

MATERIALS AND METHODS

• Materials

Microcrystalline cellulose (MCC) (Avicel PH 105, FMC, USA) was used as filler and starting material. Polyethylene glycol (PEG) 4000 (Clariant GmbH, Germany), poloxamer 188 (Lutrol F68, BASF, Germany) and Gelucire 44/14 (Gattefosse, France) were used as meltable binders. PEG 400 (Fluka, Germany) was added to reduce the friability of microparticles when PEG 4000 was used as binder. Lactose NF mesh 200 (DMV International, Netherlands) was used in preparation of reference clarithromycin granules. Clarithromycin (CLA) was supplied by Lek, Slovenia.

• Methods

Starting materials used as binders were characterized by differential scanning calorimetry (DSC), either alone or in a 1:1 physical mixture with the CLA. DSC measurements were done on a Pyris 1 DSC (Perkin Elmer, USA).

Samples were prepared in a modified Glatt GPCG-1 apparatus (Glatt, Germany), equipped with a rotator granulator insert. The apparatus was enclosed in a custom built insulating housing and the nozzle outlet was encased in a custom built polytetrafluoroethylene casing.

Batches of 300 g of a mixture of CLA and MCC in ratio 1:1 were used in each experiment. Prior to preparation of microparticles the apparatus was heated to a set temperature. Binder material was molten and thermostated to a set temperature during the process. Binder melt was sprayed at a constant rate onto the fluidized powders at a constant rotor speed. All key parameters (inlet air temperature, starting chamber temperature, spray rate, atomizing air pressure and atomizing air temperature) were optimized and set for individual binder. Spheronization of the particles was performed in some cases at the starting inlet air temperature.

Prepared particles were characterized by sieve analysis, friability test, optical evaluation, and in vitro dissolution in phosphate buffer pH 6.8 at 37 ˚C.

Reference granules with CLA were prepared by wet granulation. Meltable binders were replaced with lactose. Equal amounts of CLA and MCC were mixed with lactose to obtain a composition containing 40% CLA. The composition of the prepared mixture corresponded to the CLA content in microparticles prepared with the hot-melt method.

RESULTS AND DISCUSSION

Thermograms of dispersions of CLA in binders are useful to predict possible interactions between these substances. Since there were no significant changes in DSC curves for dispersions of CLA in binders

compared to pure binders, it was assumed that there are no significant interactions between CLA and binders which could influence temperature behavior of binders (e.g. melting point).
When PEG 4000 was used as a melted binder, the resulting microparticles exhibited high friability. Therefore a mixture of PEG 4000 and PEG 400 in the ratio 22:3 (wt/wt) was used. The limiting factor of the process was bed temperature - when the bed temperature increased above 52 °C, uncontrolled agglomeration occurred.
Spheronization of microparticles did not decrease the friability. Optical examination of microparticles revealed that spheronization increased the surface smoothness of microparticles but it did not increase their sphericity.
From the experiments conducted with PEG as binder we can conclude that higher temperature of inlet air and higher starting temperature in the process chamber decreases product friability. On the other hand, with the increase in these two factors the maximum amount of binder melt that can be applied on the powders decreases. With decreasing amount of applied binder, friability increases due to insufficient binding of powder particles. Consequently, to achieve optimal friability and final characteristics of the product, a careful balance of these three factors is needed.
Using Gelucire 44/14 as binder, the limiting factor of the process was bed temperature - when the bed temperature rose above 42 °C, a quick and widespread agglomeration occurred. Due to its predicted low influence on final product characteristics, spheronization was not performed. Optical examination of product revealed irregular shape of particles.
Decrease of binder amount and droplet size did result in much higher useful yield, but on the other hand greatly increased the friability of the product.
Compared to previous experiments, the use of poloxamer 188 melt as binder did not limit the process with certain bed temperature, although it was predicted that agglomeration would occur if the temperature was allowed to rise higher than the maximum tested temperature (57°C). Particle growth was more constant and did not exhibit the sudden agglomeration that were seen with PEG and Gelucire 44/14 melts, but nevertheless the particle size distribution was wide. Size distribution could not be narrowed by optimization of tested variables like inlet air temperature, starting chamber temperature, spray rate or spheronization.
Optical analysis showed that spheronization improved microparticle sphericity and surface smoothness. The amount of binder melt applied, inlet air temperature, starting chamber temperature and spray rate showed little influence on friability. Spheronization, however, was found to reduce friability.
Total yield of the process and friability of microparticles prepared with PEG 4000, Gelucire 44/14 and Poloxamer 188 at optimal process parameters are shown in Table 1.

Table 1: Total yield and friability microparticles prepared with different binders.

Binder	Total yield (%)	Friability (%)
PEG 4000/PEG 400	88.8	4.70
Gelucire 44/14	79.5	9.32
Poloxamer 188	87.9	4.57

Dissolution rate of CLA from prepared microparticles was increased in comparison to reference granules prepared by wet granulation. Microparticles prepared with the hot-melt method showed immediate release of CLA with more than 80% of active substance released in the first 15 minutes and complete release after 60 minutes.

CONCLUSIONS

Bed temperature was shown to be the major limiting factor for hot melt microparticles preparation in a rotary fluid bed apparatus. Polymers with lower melting temperatures were therefore less suitable for microparticles preparation. The dissolution rate of CLA was greatly increased in the microparticles prepared with meltable binders, as compared to reference clarithromycin granules. We can conclude that poloxamer 188 is the most promising binder for preparation of CLA-loaded microparticles, taking into account process stability and microparticles characteristics.

References

1. M.J. Jozwiakowski, D.M. Jones, R.M. Franz, Characterization of a hot-melt fluid bed coating process for fine granules, Pharm. Res. 7 (1990) 1119-1126.
2. T. Vilhelmsen, H. Eliasen, T. Schaefer, Effect of a melt agglomeration process on agglomerates containing solid dispersions, Int. J. Pharm. 303 (2005) 132-142.

PO030

RP-HPLC method for determination of *lenograstim* (rHuG-CSF) in pharmaceutical formulation

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INTRODUCTION

Human granulocyte colony – stimulating factor (HuG-CSF) is one of the hematopoietic growth factors which plays an important role in hematopoietic cell proliferation, differentiation of hemopoietic precursor cells and activation of mature neutrophilic granulocytes. With the recent increased understanding of its biological properties *in vivo* together with available preparations of highly purified recombinant human G-CSF, this growth factor has become an essential agent for clinical applica-

tions. It is used widely to reduce the risk of infection that occurs in neutropenic patients as a result of an aggressive cytotoxic chemotherapy or after bone marrow transplantation [1].

Two forms of rHuG-CSF are currently available for clinical use: non-glycosylated protein - filgrastim and glycosylated protein - lenograstim. Lenograstim produced in Chinese hamster ovary (CHO) cells is a 19,6 kDa glycoprotein consisting of 174 amino acids and possesses an *O*-linked carbohydrate chain attached to Thr-133 of the molecule. Lenograstim is indistinguishable in structure and activity from natural human (endogenous) G-CSF [2].

Glycosylation has been reported to confer many advantages over non-glycosylation, such as greater stability against heat denaturation and pH and resistance to proteolytic degradation [3]. In addition, lenograstim exhibits higher biological and pharmacological potency compared with filgrastim [4, 5].

The aim of our study was to develop a simple and sensitive RP-HPLC method for identification and determination of lenograstim (glycosylated form of rHuG-CSF) in pharmaceutical formulations and to validate the method for linearity, range, precision, accuracy, limit of quantitation, limit of detection and robustness following the ICH guidelines [6].

Experimental

Chemicals and reagents

The lenograstim reference material was supplied by Ray Biotech (USA). Lenograstim sample preparations containing 263 μg per ml were obtained from commercial sources. All chemicals were of a HPLC grade. For all analyses HPLC-grade water was used.

Apparatus and chromatographic conditions

A Waters HPLC system was used equipped with
a Waters 600 pump, Waters 996 photodiode array detector and Millennium 32® software for data handling.

The experiments were carried out by reverse-phase HPLC using LiChrospher® WP 300 RP-18e, (150 x 4 mm i.d.) column. The mobile phase was consisted of solvent A (0,1% TFA in water) and solvent B (acetonitrile). The column was initially equilibrated with 40% B and eluted by linear gradients from 40% B to 60% B over 10 minutes at a flow rate of 1 ml/min and from 60% B to 70% B over 20 minutes at a flow rate of 0.6 ml/min. The temperature was 50°C and UV detection was set at 215 nm. Samples were injected through Rheodyne injector valve with 200 μL sample loop.

Standard and sample solutions of lenograstim

The standard and sample solutions of lenograstim were prepared by dissolving in water to the working concentration of 50 μg mL^{-1}.

Linearity and range

The calibration curve was constructed with five standard concentrations of lenograstim in the range of 12,5 -100 μg mL^{-1}.

Precision

Repeatability of the method was determined by performing the analytical procedure six times using the samples at the same concentration. Inter-day precision was assessed by comparing the results obtained from six quantitative determinations on three different days.

Accuracy

The accuracy of the proposed method was assessed by performing a total of nine determinations of three concentration levels covering the specified range.

Limit of quantitation and limit of detection

The LOQ and LOD were obtained from the slope of the calibration curve and the standard deviation of the response, determined by a linear regression line as defined by ICH.

Robustness

The robustness of the RP-HPLC method performance was determined after slightly varying the experimental conditions from optimized values (column temperature, composition of mobile phase and wavelength).

RESULTS AND DISCUSSION

Linearity and range

The calibration curve was constructed from the peak areas *versus* concentration; this was found to be linear in the 12,5-100 μg mL^{-1} range. A linear regression by the least squares method was applied.
a= 10.22; b=310.05; r^2= 0,9999
The high value of the determination coefficient indicated good linearity of the method.

Precision

The indicator of precision of the method is the relative standard deviation (RSD ≤ 2.0%).

Table 1: Repeatability and inter -day precision data of RP-HPLC for lenogratsim

	Theoretical μg mL^{-1}	Determined μg mL^{-1}	RSD %
Intra-day (n = 6)	263	263.96	0.68
Inter-day (n = 18)	263	263.88	0.54

The obtained RSD values for repeatability and inter-day precision were 0.68% and 0.54%, respectively.

Accuracy

The accuracy was confirmed from triplicate determinations of three different solutions containing 25, 50 and 75 μg mL^{-1} lenograstim.

Table 2: Accuracy of RP-HPLC for lenogratsim

Theoretical μg mL^{-1}	Determined μg mL^{-1}	Determined* μg mL^{-1}	Recovery %
25	24.82	261.12	99.27
50	50.18	263.96	100.36
75	75.35	264.23	100.46

n = 3, *expressed as nominal concentration

The obtained recovery values of 99.27%, 100.36% and 100.46% for each concentration, indicate that the propose RP-HPLC method is quantitative and accurate.

Limit of quantitation and limit of detection

The LOQ and LOD were found to be 3.93 μg mL^{-1} and 1.29 μg mL^{-1}, respectively.

Robustness

Table 3: Robustness testing

Variable	Optimized value	Investigated value
Column temperature	50 ˚C	40 ˚C and 50 ˚C
Composition of mobile phase	0.1% *V/V*TFA/H_2O +AcN	0.1% *V/V*TFA/H_2O +AcN; + 0.01% *V/V*TFA/AcN
Wavelength nm	215	210- 220

There were no significant changes in the chromatographic pattern when the above modifications were made in experimental conditions, thus showing the method to be robust.

The chromatograms of standard solution (a) and sample solution (b) of lenograstim are presented in Fig. 1.

CONCLUSION

In this study, a simple and sensitive RP-HPLC method for identification and quantification of lenograstim in pharmaceutical formulations was developed. The validation data shows that the proposed method is accurate and robust and possesses excellent linearity and precision characteristics. This RP-HPLC method can readily be used in the routine analysis of lenograstim in pharmaceutical formulations.

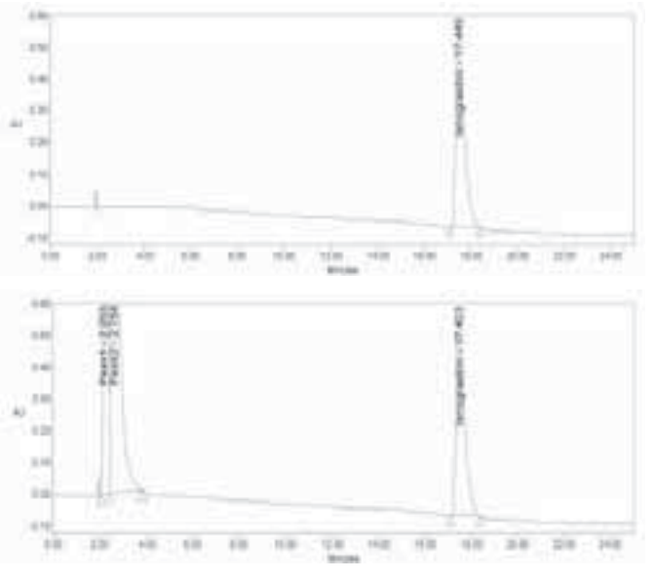

Figure 1: Chromatograms of: a) Standard solution of lenograstim (50 μg mL^{-1}); b) Sample solution of lenograstim (50 μg mL^{-1})

References

1. A. C. Herman et al., *Formulation, Characterization and Stability of Protein Drugs*, Plenum Press, New York 1996, pp. 303-328.
2. G. Zhou et al., *J. Pharm. Bio. Analysis* **35** 425-432 (2004)
3. V.Gervais et al., *Eur.J. Biochem.* **247** (1997) 386-395.
4. A. Hüttmann et al., *J. Cancer. Res. Clin. Oncol.* **131** 152–156 (2005)
5. M. Hoglund et al., *Eur. J. Haematol.* **59** 177-183 (1997)
6. International conference on harmonisation (ICH) of technical requirements for the registration of pharmaceutical for human use. Validation of analytical procedures: Text and Methodology (Q2(R1)), 1-13 (2005)

PO031

Development of a new HPLC method for the quantitive determination of levothyroxine sodium released from tablets

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INTRODUCTION

Levothyroxine sodium (LTS) is the sodium salt of the levo isomer of the thyroid hormone thyroxine. Thyroid hormones affect protein, lipid, and carbohydrate metabolism, growth, and development. They stimulate the oxygen consumption of most cells of the body, resulting in increased energy expenditure and heat production, and possess a cardiostimulatory effect that may be the result of a direct action on the heart [1].

The concentration of LTS in dissolution studies is typically measured using HPLC methods. USP 30 introduced four different dissolution tests. Test 1 specifies 0.01 mol/l hydrochloric acid containing 0.2% sodium lauryl sulphate (SDS) as a medium [2].

SDS is an anionic surfactant which is accumulating on HPLC column, causing pressure increase, peak shape and symmetry change thus disrupting determination of LTS. This phenomenon is especially outstanding if a large volume of the test solution is injected (800 μl). To overcome this problem some authors proposed different analytical methods for determination of LTS released from tablets [3, 4].

The purpose of this study was to develop a new, accurate, sensitive and reproducible HPLC analysis method for determining LTS released from immediate release tablets in the medium containing 0.2% SDS suitable for routine control.

MATERIALS AND METHODS

• Materials

The following materials were used: LTS (Peptido, Germany), tetrabutylammonium hydrogensulphate p.a. (Merck, Germany), acetonitrile HPLC grade (JT Baker, Netherlands), SDS p.a. (Merck, Germany), hydrochloric acid (Merck, Germany).

• In vitro release testing

The in vitro release tests were performed using the paddle method in 500 ml of 0.2% SDS in 0.01 mol/l hydrochloric acid solution maintained at 37 ± 0.5°C. The paddle rotation speed was kept at 50 rpm. The samples were assayed after 45 minutes, filtered through a glass mikrofibre filter paper and injected into the HPLC system [2]. The tests were performed protected from light.

• HPLC analysis method

The amounts of released LTS were determined by HPLC method on the chromatographic system Hewlett Packard, series 1100 with UV/VIS-detector. The separation was achieved on Zorbax® column SB-C18 (250x4.6mm, 5 μm). The amounts of 100 μl of each of the standard and test solution were injected onto the column and eluted with the mobile phase A - aqueous solution containing 0.34% w/V tetrabutylammonium hydrogensulphate and the mobile phase B - acetonitrile (according to the elution programme shown in Table 1) during 25 minutes. Detection was accomplished using a variable UV/VIS detector set at 225 nm wavelength.

Table 1: The elution programme.

time (min)	mobile phase A (%)	mobile phase B (%)	flow rate (ml/min)
0 – 10	65	35	1.0
10 - 11	65 → 30	35 → 70	1.5
11 - 18	30	70	1.5

The validation procedure, parameters and acceptance criteria were based on ICH and FDA guidelines and recommendations [5-7]. The method was validated in terms of linearity, accuracy, specificity, limits of detection and quantification and precision.

The concentration of the standard solution, prepared by dissolving the LTS reference standard into the release medium, was 0.2 μg/ml. Stability of the standard and test solutions was were monitored during 24 hours.

In order to investigate the applicability of the method for long-term, routine control, the retention time, shape and peak symmetry of LTS, as well as the column pressure were tested during 16 hours.

RESULTS AND DISCUSSION

Figure 1. represents the selectivity of analytical method by comparing UV spectra of the placebo solution with those obtained with the standard and test solutions.

System suitability was confirmed by calculating the tailing factor and relative standard deviation (RSD) for five consecutive injections of the standard solution. The obtained values were 0.3% and 1.1, respectively. Linear relationship was obtained in the range from 0.1 μg/ml to 0.3 μg/ml with the correlation coefficient of 0.99997.

The obtained results indicated good stability of the standard and test solutions during 18 hours (the changes in peak area were less than 2%), which is very important considering the duration of one analysis (25 minutes). The other tested parameters were within specification limits.

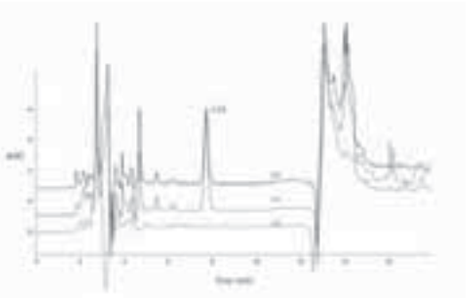

Figure 1: Chromatograms of placebo (1), standard (2) and test solution (3), respectively.

Chromatograms obtained during 16 hours indicated that, even after numerous injections of tested solutions, no changes were observed in shape and symmetry of LTS peak (Figure 2).

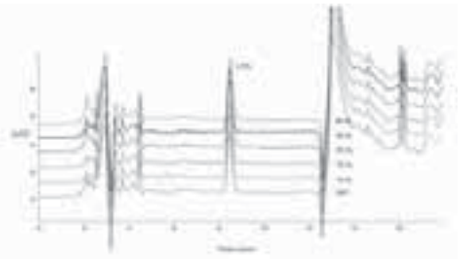

Figure 2: Chromatograms of test solutions continuously analyzed during a 16-hour period.

CONCLUSION

The proposed HPLC method is very suitable for assessing the LTS released from tablets in the medium containing 0.2% sodium lauryl sulphate, at the early stages of formulation development, during the optimisation process, for stability studies, and routine quality control of the batches. The procedure enables continuous evaluation of a great number of samples, without problems associated with column pressure increase and peak deformity.

REFERENCES

1. Martindale, The Extra Pharmacopoeia, 35th ed., The Pharmaceutical press, London, 2007.
2. The United States Pharmacopoeia, 30th edition, United States Pharmacopeial Convention, Inc., Rockville, 2007.
3. Gika HG, Samanidou VF, Papadonoyannis IN., J. Chromatogr., 2005; 814: 163.
4. Pabla D, Akhlaghi F, Ahmed A, Zia H, Rapid Commun. Mass Spectrom. 2008; 22: 993-996.
5. ICH Guidelines Q2A: Text on Validation of Analiytical Procedures: Definitions and Terminology, 1994.
6. ICH Guidelines Q2B: Text on Validation of Analiytical Procedures: Methodology, 1996.
7. FDA Guidance for Industry: Analiytical Procedures and Method Validation, 2000.

PO032

Chemometrical approach in clarithromycin and its related compounds analysis by rapid resolution RP-HPLC method

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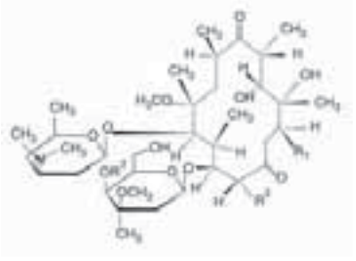
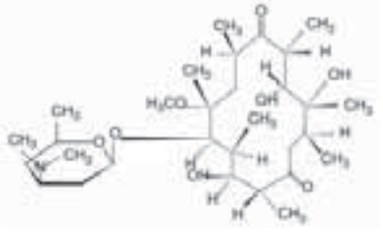
INTRODUCTION

Clarithromycin is a semisynthetic macrolide derived from erythromycin A and consists of a 14-membered lactone ring as well as cladinose desosamine residues at positions 3 and 5 of the ring, respectively. At very low pH, it degrades rapidly [1]. For Clarithromycin, five related compounds are specified as impurities that can appear in the drug substance.(Fig.1)



Clarithromycin (6-O-methyleritromycin A)
KLN 3: R1=CH3, R2=R3=H
KLN 5: R1=R2=CH3, R3=H
KLN 7: R1=R2=R3=CH3
KLN 8: R1=R3=CH3, R2=H



KLN1: 3-O-decladinosyl-6-O-methyleritromycin

Figure 1: Chemical structure of clarithromycin and related compounds

A literature search has shown HPLC method for the determination of macrolide antibiotic, including claritromycin, [2] and HPLC methods for determination of clarithromycin and its related compound KLN5 [3,4]. This manuscript describes the development and validation of a Rapid Resolution HPLC method for simultaneous determination of clarithromycin and its impurities using Design of Experiments (DoE) approach.

EXPERIMENTAL SECTION

- Agilent RR HPLC system, 1200 series, (Binary pump SL, Diode Array Detector SL, Column compartment TCC SL) High Performance Autosampler SL (HIP-ALS SL)
- Zorbax Eclipse XDB C18 Rapid Resolution High-Throughput (RR HT) 4.6 mm 50 mm, 1,8 μm particle size column
- Mobile phase containing acetonitrile (ACN) and phosphate buffer pH 7,0 in different (v/v) ratios was used
- A single UV absorbance was measured at 205nm.
- The peak areas were integrated automatically with Windows NT based LC ChemStation Software.
- The MatLab software was used for generation and evaluation of the experimental design.

TEST SOLUTION

Test solution was prepared by dissolving the respective working standard substances in a mixture of ACN : phosphate buffer pH 4,5 (40:60, v/v). For the screening experiments and optimization procedure the laboratory mixture of clarithromycin and impurities in the ratio which corresponded to the recommendation by the ICH, Topic Q3A(R2) was used [5]. Laboratory mixture of 100 μg ml^{-1} clarithromycin and 0,05 μg ml^{-1} of each related compound was prepared.

Screening Experimental design

- Full factorial design 2^3 (second order interaction model), was used as a screening design (to identify influence of all factors and its interactions on the conditions of separation)

$$y = b_o + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{123} x_1 x_2 x_3$$

Table 1: Cosidered factors

		Factor levels		
	Factors	-	+	0
x_1	Organic phase variation during gradient (v/v, %)	25-45	25-75	25-60
x_2	Column temperature ˚C	40	60	50
x_3	Gradient rise time (min)	5	12	7

- Rs values for all consecutive peak pairs were applied to estimate coefficients of the linear model.

OPTIMIZATION

The screening experiments have shown that the separation of the investigated substances as measured by the Rs values is most affected by the organic phase variations during gradient elution (factor x_1) and column temperature (factor x_2). In the second stage these two most important factors were simultaneously varied. Resolution (Rs) of obtained peak pairs were estimated for a combination of five values of col-

umn temperature (40, 45, 50, 55 and 60 °C) and six basic gradients, where ACN content in the mobile phase was varied from 25 to 45%, from 25 to 50%, from 25 to 55%, 25 to 60%, 25 to 65% and from 25 to 67%, at gradient rise time of 7 min. CRF values were calculated for these 25 experiments.

- RSM (Response surface methodology) was applied for determination of an optimium set of relevant experimental chromatographic factors for separation of impurities from drug substances. Experimental data was fitted in a full quadratic 22 responce surface model;

$$y = b_o + b_1 x_1 + b_2 x_2 + b_{11} x_{12} + b_{22} x_{22} + b_{12} x_1 x_2$$

- The separation quality of clarithromycin and releated compounds for achieving the maximum assay time was assesed dy calculating the value of Chromatografic Response Function (CRF)

$$CRF = \prod_{i=1}^{L-1} R_s\,(i, i+1)$$

R_s = the resolution between peak No. i and peak No. i+1

- Minimum obtained value of individual Rs values of 1.5 as a selection criterion was used
- Contour diagram of the CRF was constructed

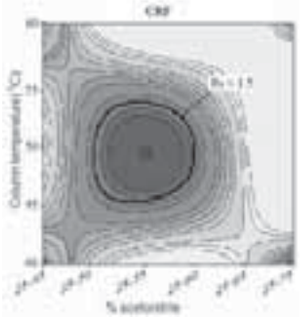


Figure 2: Contour diagrams of the CRF. The contour corresponding to all Rs-values ≥ 1.5 is marked.

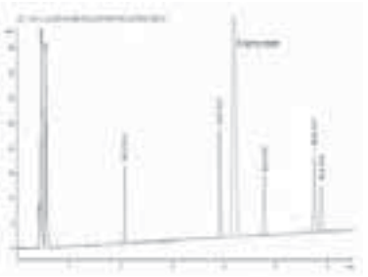


Figure 3: The chromatogram of test solution (organic phase variation during gradient elution from 25 to 55 %, v/v, gradient rise time of 7 min, flow rate 0.9 ml/min, column temperature 50 °C).

CONCLUSION

The proposed Rapid Resolutin HPLC method permits simultaneous determination of clarithromycin and its related compounds specified as impurities due to good separation and resolution of the chromatographic peaks. The amount and purity of the active substance, the percent level of impurities, and a total chromatographic purity can be determined in a single analysis. The linear models obtained demonstrate a large influence of organic phase variation during gradient elution and column temperature to the resolution between investigated substances. Valuable information about retention modeling was obtained by RSM, using resolution as important component of CRF. The methodology proposed represents an efficient and easily accomplishable approach in resolving the problem of searching for optimum HPLC conditions.

REFERENCES:

1. S. Omura (Ed), Macrolide antibiotics: chemistry, biology and practice, Academic Press, Orlando, FL, 1984.
2. C. Leal, R. Codony, R. Compano, M.Granados, M. D. Prat, J.Chromatogr. A, 910, 285–290 (2001).
3. P.O. Erah, D.A. Barrett, P.N. Shaw, J.Chromatogr. B, 682, 73 -78 (1996).
4. A. Pappa-Louisi, P. Agrafiotou, Chromatographia 55, 541-547 (2002).
5. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2006. Topic Q3A(R2), Impurities in New drug substances.

PO033

Determination of risperidone and its active metabolites (9-hydroxy-risperidone enantiomers) in human blood plasma

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INTRODUCTION

Risperidone (RISP) is an atypical antipsychotic agent. In human, it undergoes extensive alicyclic hydroxylation at position 9, leading to two active 9-hydroxyrisperidone (9-OH-RISP) enantiomers. It was suggested that the formation of 9-OH-RISP might be stereoselective, revealing that CYP2D6 could have predominant role in formation of major *(+)*-9-OH-RISP metabolite, while CYP3A4 could be involved in formation of minor *(–)*-9-OH-RISP metabolite [1]. However, in a later study the stereoselective role of CYP2D6 and CYP3A4 was questioned [2]. Additionally, large interindividual variability in RISP and 9-OH-RISP

steady state plasma concentrations was reported. Due to their equipotent pharmacological activities the sum of RISP and 9-OH-RISP plasma concentration was presented as the "active moiety concentration", which was used to establish the relationship between plasma concentration and clinical effect.

Numerous HPLC analytical methods based on UV detection, electrochemical (EC) detection or mass spectrometry (MS) have been developed to quantify RISP and 9-OH-RISP plasma concentrations. However, only one validated HPLC analytical method for enantioselective determination of 9-OH-RISP enantiomers has been reported in the literature. The analytes were determined in sub-ng-per-mL level using Chiralcel OJ chiral column in a normal phase mode and MS/MS detection [3].

We developed and validated a simple HPLC-ECD method with sufficient sensitivity for simultaneous determination of steady state plasma concentration of the three analytes of interest; *(–)*-9-OH-RISP, *(+)*-9-OH-RISP, and RISP. The method was successfully applied in a clinical study investigating the large interindividual varability in RISP pharmacokinetics and stereoselectivity of 9-OH-RISP formation.

EXPERIMENTAL

Chromatographic system

The Agilent 1100/1200 Series HPLC system (Agilent Techologies Inc.) consisted of vacuum degasser, quaternary pump, thermostated autosampler (5 °C), and thermostated column compartment with valve switching device was controlled by Agilent ChemStation software program version A.09. The CoulArray electrochemical detector equipped with four sensor analytical cell (ESA Inc.) was coupled to the HPLC system using the valve switching device. The separation of 9-hydroxyrisperidone enantiomers was achieved on Chiral-AGP analytical column at 25 °C. The isocratic mobile phase consisted of a mixture (15:85 vol/vol) of methanol and phosphate buffer (100 mM, pH 6.2), respectively. The mobile phase flow rate in the first 4 minutes of analysis run time was maintained at 0.8 mL/min. Between 4.0 and 11.0 minutes the flow rate was constantly increasing from 0.8 to 0.9 mL/min. The total analysis run time was 11 minutes. After each sample analysis had finished the mobile phase flow rate was set back to 0.8 mL/min. The potentials applied to the analytical cell were 500, 650, 950, and 950 mV, for electrodes 1 to 4, respectively. The column system was connected to ECD, except for the time period from 0.5 to 2 minutes after the sample injection.

Solid phase extraction (SPE) procedure

The analytes were extracted from plasma using SPE cartridges with weak cation exchange sorbent: Strata-X-CW 60 mg (Phenomenex Inc.). The cartridges were placed on a vacuum manifold connected to a water aspirator. After sorbent conditioning (2mL of methanol) and equilibration (2 mL water), 1.0 mL of plasma sample plus 100 μL of IS working solution and 2 mL of sodium acetate buffer (100 mM, pH 4.5) was loaded into the cartridge. The cartridge was washed with 2 mL of sodium acetate buffer (100 mM, pH 4.5) and 2 mL of methanol, successively. After drying the sorbent by increasing the vacuum inside the basin, the analytes elution was achieved with 2 mL of formic acid/methanol/acetonitrile (2/50/50 v/v/v) mixture. The eluate was dried out at 40°C under a gentle stream of nitrogen in a Turpovab apparatus (Zymark Inc.). The dried samples were reconstituted in 200 L of mobile phase and 50 μL was injected into the HPLC system.

Standard solutions

The fenoterol (internal standard - IS) solution was freshly prepared on a daily basis. The standard plasma samples used for calibration contained 2, 5, 15, 50, 100, and 200 ng/mL of racemic 9-OH-RISP and 1, 2, 5, 15, 50, and 100 ng/mL of RISP. The concentrations of each of the 9-OH-RISP enantiomers were set at half of the concentration of the 9-OH-RISP. Three-level quality control plasma samples (QC) were prepared. All spiked samples were stored at –20°C.

RESULTS

The analytes were baseline separated in the following order; IS (3.3 min), *(–)*-9-OH-RISP (4.9 min), *(+)*-9-OH-RISP (6.5 min), and RISP (9.0 min) - Figure 1. The column system was not connected with ECD during first 2 min, therefore, the chromatograms attained from ECD data were not recorded until 2.5 minutes after the sample injection.

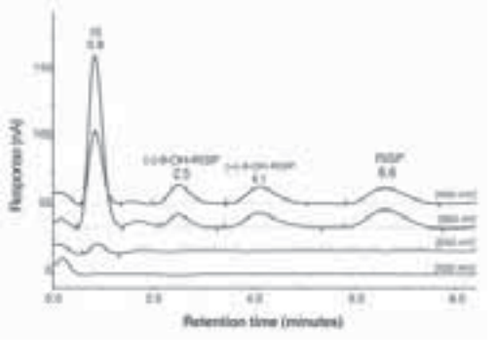

Figure 1: A typical chromatogram obtained after spiking medium quality control sample. RISP and both 9-OH-RISP enantiomers concentrations were 10.2 and 6.8 ng/mL, respectively.

Selectivity, detection limit, linear range, intraday repeatability and accuracy, interday accuracy and precision, extraction recovery and post-preparative stability were determined according to the FDA guidance (Table 1).

Table 1. Summary of method validation parameters.

Validation parameter		*(–)*-9-OH-RISP	*(+)*-9-OH-RISP	RISP
Intraday repeatability (%, n=3)	Lo	4.8 - 6.4	4.6 - 7.9	3.1 - 13.9
	Me	4.9 - 7.7	4.8 - 8.9	2.4 - 4.5
	Hi	2.6 - 6.4	2.9 - 5.7	3.7 - 9.5
Interday accuracy (%) and precision (%, n=3)	Lo	103.7 (2.8)	104.5 (7.0)	106.2 (7.2)
	Me	95.1 (6.6)	98.1 (0.5)	97.1 (6.5)
	Hi	96.5 (3.2)	99.0 (0.2)	104.6 (5.2)
Post-preparative stability (%, n=3)	Lo	98.4	101.6	105.2
	Me	94.2	98.3	101.6
	Hi	92.5	94.8	101.1
Extraction recovery (%)		86	91	96
Detection limit (ng/mL)		0.50	0.50	0.50
Linear range in ng/mL		1.0 - 100	1.0 - 100	1.0 - 100

RSD; relative standard deviation, Lo, Me, Hi; low, medium, and high quality control plasma samples, respectively.

CONCLUSION

A simple HPLC method with ECD detection and SPE extraction procedure was developed and validated for simultaneous measuring of *(–)*-9-OH-RISP, *(+)*-9-OH-RISP, and RISP plasma concentration in patients on risperidone therapy. Present validation data demonstrate that the method is consistent and reliable with low values of R.S.D. and bias. The analytes detection limits (0.5 ng/mL) were low enough to measure steady state plasma concentrations of these three analytes in patients on risperidone therapy.

References

1. Yasui-Furukori N et al. Different enantioselective 9-hydro-xylation of risperidone by the two human CYP2D6 and CYP3A4 enzymes. Drug Metab Dispos, 2001; 29(10): 1263-1268.
2. Yasui-Furukori N et al. Effects of CYP2D6 genotypes on plasma concentrations of risperidone and enantiomers of 9-hydroxy-risperidone in Japanese patients with schizophrenia. J Clin Pharmacol, 2003; 43(2): 122-127.
3. Cabovska B et al. Determination of risperidone and enantiomers of 9-hydroxyrisperidone in plasma by LC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci, 2007; 852(1-2): 497-504.

PO034

Solubility determination of a drug by laser diffraction method

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INTRODUCTION

Classical approaches for solubility determination are based on the saturation shake-flask method. Solubility measurement at a signle pH under equilibrium conditions is largely labor-intensive but straightforward procedure, requiring long equlibration times. The analyte is added to a standard buffer solution until saturation occurs, indicated by undissolved excess drug. The thermostated saturated solution is shaken as equilibration between the two phases establishes, After microfiltration or centrifugation, the concentration of the substance in the supernatant solution is determined by, e.g., spectroscopic methods [1].

Lindfors et al [2] have investigated an alternative method for determining the solubility, based on light scattering from colloidal drug nanosuspensions. In the analyte addition method, colloidal particles are gradually added until turbidity is detected. The scattered intensity then increases significantly and, initially, linearly with the particle concentration, and the solubility is determined from the onset of intensity increase [2].

In present study we have investigated another alternative method of the solubility determination of API with laser diffractometry. Laser diffractometry is primarly used for measuring particle size and particle size distribution. The basic idea to determine solubility of API in a solvent is to measure the volume concentration which is calculated from the Beer-Lambert law and expressed as a percentage (%Vol). The measured volume concentration is in a positive correlation with particles concentration in a solvent. When particles are added to a solvent, they will at concentrations below the solubility dissolve and %Vol is measured 0 %. As the concentration is increased above the solubility, however, the solution is saturated with API and the added particles no longer dissolve. The measured %Vol should increase significantly and, initially, linearly with the particles concentration, and the solubility could be determined from the onset of intensity increase.

EXPERIMENTAL METHODS

Materials: K-1206 was supplied by Krka d.d. and used as model drug compound. K-1206 is practically insoluble in water but is sparingly soluble in alcohol (ethanol 96%).

True density of the powdered K-1206 was measured by helium pycnometer AccuPyc 1330 (Micromeritics, USA). About 1,5 g of the API was put into measuring chamber and the true density of the drug powder was measured.

Refractometer Carl Zeiss Jena J78 (Carl Zeiss inc., Germany) was used to measure **refractive index** (RI) of different API concentrations in alcohol. Refractive indices for known solute concentrations were extrapolated to 100% concentration in order to calculate the bulk RI [3]:

$$RI_{bulk} = k * 100 + a$$

where k is the slope of the function Refractive index/ K-1206 concentration, and a is obtained from the y-intercept and represents the refractive index of the solvent.

Volume concentration was determined at room temperature with a laser diffraction particle size analyzer (Malvern Mastersizer, Malvern Instruments Limited, UK). 100 mL of alcohol or 100 mL of a mixture of 1:1 purified water and alcohol were poured into Malvern Small Volume Dispersion Unit (Malvern Instruments Limited, UK) to measure the background. Then small amounts of K-1206 were successively added directly into solvent. At each addition of the drug the dispersion was equilibrated for at least 30 minutes after which volume concentration by laser diffraction measurement was recorded.

RESULTS AND DISCUSSION

True density of K-1206 was measured 1,2775 g/cm^3.

The RIs of K-1206 solutions over a range from 0.06 wt% to 2.42 wt% are reported in figure 1. As can be seen, there is a linear dependence of refractive index with concentration within the region studied. By using the equation of the best fit line (which has a 99% confidence interval) the RI at 100% concentration can be calculated. This yields an RI of 1.5318.

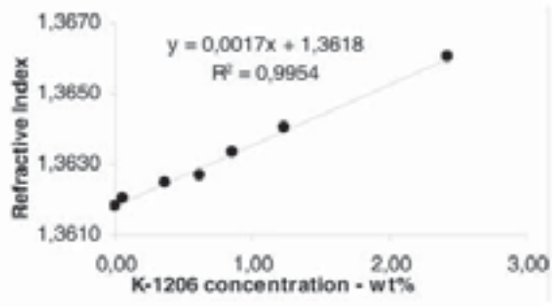

Figure 1. Refractive indices of K-1206 solutions.

Solubility of the K-1206 in alcohol and in 1:1 mixture of purified water and alcohol

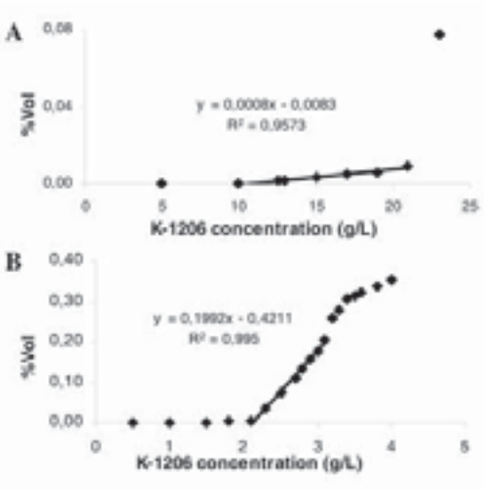

Figure 2: Measured %Vol vs K-1206 concentration in (A) alcohol and (B) mixture of 1:1 water and alcohol. The solid lines are linear fit to certain regions of concentration data.

The solubilities were evaluated from the experiments in figure 2, panels A and B, by fitting the certain region of concentration data to a straight line. With this fitting we obtain x-intercepts, which represent the solubilities of API in solvents. With this procedure we obtain solubility of K-1206 in alcohol 10.4 g/L and in 1:1 mixture of purified water and alcohol 2,1 g/L.

It should be noted that linearity was obtained only in a certain region of concentration data. This was due to high obscuration at high concentration data which disabled accurate measurement. Too much sample causes the light scattered from an individual particle to be scattered itself by other particles – this is known as multiple scattering [4].

CONCLUSION

Simple refractometer measurements provide a rapid, reliable method of calculating the RI of compounds such as K-1206 to an accuracy that is needed for laser diffraction measurement. By determining the true density and RI of powdered K-1206 there is no need to rely on Fraunhofer Approximation, allowing users to avoid the errors associated with its use [4].

Solubility is an important factor for particle design of a drug when nonsolvent method is used for precipitaton. For experimental purposes of particle design drug solution is prepared in solvent in such concentration that it precipitates after addition of nonsolvent. For that matter it is crucial to determine the solubility of a compound in solvents which are used for drug design techniques. Results of this investigation show laser diffractometry could be a good and rapid method for measuring solubility of API. With the laser diffraction technique the solubility experiments can be extended also to higher temperatures without any complications.

References:

1. Avdeef A, Testa B. Cell. Mol. Life Sci. 2002; 59, 1681-1689.
2. Lindfors L et al. Langmuir 2006; 22, 911-916
3. Saveyn H et al. Particle and Particle Systems Char. 2002; 19, 426-432.
4. Malvern Mastersizer »Getting Started« manual 1997; 3.5, 5.1-5.5

PO035

Density functional theory calculations of the interaction between β-cyclodextrin and single protic solvent molecules

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INTRODUCTION

The solvent plays a very important role for inclusion complexation of cyclodextrins, as first of all there is a competition between the solvent molecules and the guest molecules, and, moreover, the equilibrium constant of the host-guest association depends to a high extent on the properties of the solvents. In aqueous solution containing cosolvents, the equilibrium constants and the related thermodynamic parameters change significantly even on small variations of the concentration of the cosolvent [1,2].

In order to get more insight into the interaction of solvent molecules with β-cyclodextrin (β-CD), the 1:1 complexes of β-CD and water, and the protic solvent molecules methanol and ethanol were studied by Density Functional Theory (DFT) calculations. Particularly, hydrogen bonding between the solvent molecules and the primary and secondary hydroxyl groups of β-CD were investigated in detail. The aim of the study was to characterize the interaction of these protic solvent molecules with different parts of β-CD.

METHODS OF CALCULATION

DFT calculations were performed using the program package GAUSSIAN03. A minimized standard geometry of β-CD was taken as starting geometry. Water, methanol or ethanol molecules were positioned systematically around the cyclodexrin rim and the geometries of the complexes were minimized. Because of the size of the association complexes, the calculations were limited to the B3LYP/6-31G(d,p) level. However, BSSE corrections were additionally applied.

RESULTS AND DISCUSSION

Many conceivable conformational minima of the CD complexes with water (and the alcohols) were determined and characterized according to their binding energies. Evidently, the protic solvent molecules can act as proton acceptor and as proton donor for the hydrogen bonds, which is shown in Figure 1.

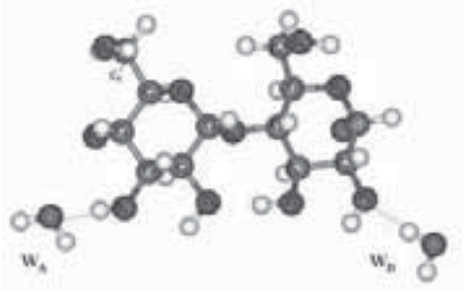

Figure 1: Numbering of the diglucose units in the β-CD rim and water presented as donor and acceptor for building the hydrogen bonds.

Some selected structures of complexes are given in Figure 2.

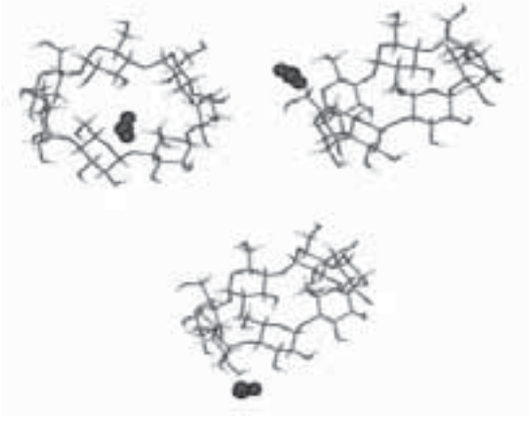

Figure 2: Geometries of various 1:1 Complexes of β-CD and water.

The calculated energies and the position of the hydrogen bonds between the protic solvents and symbol 98 \f "Symbol" \s 10-CD are given in Table 1.

The highest affinities for water and the other protic solvents were found at the secondary hydroxyl groups of the β-CD rim, where one or even two hydrogen bonds can be formed. Interactions at the primary hydroxyl groups appear to be of lower energy, and hydrogen bonding in the interior of the β-CD moiety is least favorable. Methanol and ethanol molecules show slightly lower binding affinities at the hydroxyl groups, but larger ones inside the ring.

Table 1: Position of the hydrogen bonds in different configurations and the resulting complex energies (in kcal/mol).

β-CD/ water complex	β-CD/ methanol complex	β-CD/ ethanol complex	Hydrogen bond(s)	Position of the Hydrogen bond(s)	
0.06	0.81	-0.25	none		
0.45	-1.84	00.46	none		
-1.56	-0.99	-0.71	1	O_4-W_D	
-1.1	-1.15	-1.00	1	O_4-W_D	
-1.17	-2.30	-1.40	1	O_4-W_D	
-0.97			2	O_3-W_D	O_4-W_D
-3.43	3.15	-4.98	2	O_3-W_A	$O_{2'}$-W_A
-4.24	-4.42	-3.84	2	O_3-W_A	O_3-W_D
-5.98	-6.56	-7.44	1	O_6-W_A	
-5.75	-5.74	-6.36	2	O_5-W_D	$O_{6'}$-W_A
-6.13			2	O_5-W_D	$O_{6'}$-W_A
-6.96	-7.09	-9.73	2	O_5-W_D	O_6-W_A
-8.81	8.22	-9.72	2	O_2-W_D	O_3-W_A
-9.85	-9.63	10.01	2	O_2-W_D	O_3-W_A

CONCLUSION

The highest affinities for water and the other protic solvents were found at the secondary hydroxyl groups of the β-CD rim, where one or even two hydrogen bonds can be formed. Interactions at the primary hydroxyl groups appear to be of lower energy, and hydrogen bonding in the interior of the β-CD moiety is less favorable. Methanol and ethanol molecules show slightly lower binding affinities at the hydroxyl groups, but larger ones inside the ring.

References

1. Viernstein H., Weiss-Greiler P., Wolschann P. Solubility enhancement of low soluble biologically active compounds – temperature and cosolvent dependent inclusion complexation. Int. J. Pharmaceutics 256: 85-94 (2003).
2. Viernstein H., Weiss-Greiler P., Wolschann P. Solubility Enhancement of Low Soluble Biologically Active Compounds by β-Cyclodextrin and Dimethyl-β-cyclodextrin. J. Incl. Phenom. 44: 235-239 (2002).

PO036

Feasibility testing of 96-well plate method for thermodynamic solubility determinations

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INTRODUCTION

Oral route is the most patient convenient and cost effective way to deliver drugs. Often, inadequate aqueous solubility limits the bioavailability of drugs, causing a growing concern for the pharmaceutical industry, since most of the newly developed drug candidates are poorly water-soluble [1]. The two model compounds of this study, indomethacin and piroxicam, are classified as BCS class II drugs, with poor aqueous solubility [2]. Both of them also exist in at least two different polymorphic forms, which complicates their solubility characterisation further.

High throughput screening (HTS) methods are used to measure solubilities in high numbers and small compound quantities during the early stages of drug development. Most HTS techniques determine the kinetic solubility parameters based on the detection of drug precipitation in aqueous solutions. The compound is usually dissolved in DMSO and small volumes of the aqueous dissolution media are added until the solubility limit is reached. Concentration at which the compound precipitates is defined as the solubility limit [3]. The kinetic solubility measurements are mostly used in the early stages of drug development and the solubility values may over-predict the true thermodynamic solubility values due to supersaturation.

The overall aim of this study is to develop a HTS based screening methodology for drug development. In this study we investigated two poorly soluble model drugs, indomethacin and piroxicam. To measure the thermodynamic solubility, the drugs were initially in solid form and the aqueous medium was added. As a reference we used the traditional shake flask solubility testing method.

METHODS

Indomethacin (Fluka Biochemika, Italy) and piroxicam (Hawkins Inc., CAS, USA) stock solutions were prepared in methanol and pipetted in 96-well plates. After solvent evaporation, 250 µl of the medium (water, buffer solutions of pH 1.2 or pH 6.8) [4] was added and the 96-well plates were shaken (300 rpm, 600 rpm and 1200 rpm) at room temperature (+23°C). Samples were withdrawn after 30 min, 1 h, 2 h and 5 h. Before analysis by UV plate reader (Varioskan, Thermo Electron corporation, Finland, λ=230-400 nm), the plates were centrifuged (Eppendorf AG, Germany) at 3000 rpm for 15 minutes [5].

The shake flask analyses were performed at the same conditions as the HTS method, but they lasted at least for 30 hours. The drugs were analyzed by UV-spectrophotometry (Ultrospec II, LKB Biochrom, England), XRPD (X-ray powder diffraction) (Bruker axs D8, Germany) and DSC (differential scanning calorimetry) (Mettler-Toledo, Greifensee, Switzerland).

RESULTS

For indomethacin, the new method was comparable to the shake flask method at pH 6.8. The best correlation in drug solubility values was at 1200 rpm shaking (0.365 0.025 mg/ml in shake flask vs. 0.322 0.052 mg/ml in HTS method). At pH 1.2, the methods gave different results (0.003 0.000 mg/ml in shake flask vs. 0.008 0.003 mg/ml for new method).

During the shake flask measurements, piroxicam concentration decreased after the initial higher solubility values, suggesting polymorphic changes in the solid residue. They were confirmed by the XRPD and DSC tests. At pH 6.8 after 5 hours, the solubility values of piroxicam were comparable between the two methods, but the 96-well plate method was not as sensitive to detect the polymorphic changes during the solubility process.

CONCLUSIONS

The presented 96-well plate method may provide basis for thermodynamic solubility measurements at small scale in HTS formatsolubility. Technical issues like shaking procedures and liquid handling in the 96-well plate format are crucial parameters for reliable test results.

REFERENCES

1. Radtke, New Drugs 3 77-83, 2001.
2. Amidon et al., Pharm Res 12 413-420, 1995.
3. Lipinski et al., Adv Drug Deliv Rev 23 3-25, 1997.
4. The United States Pharmacopeia and National Formulary, 26th edition, 2003.
5. Chen et al., Comb Chem High Throughput Screening 5 575-581, 2002.

PO037

New high-throughput liquid chromatography coupled with mass spectrometry method for quantification of memantine in pharmaceutical formulations

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INTRODUCTION

Memantine (1-amino-3,5-dimethyl-adamantane) is used for treatment of moderate to severe Alzheimer s disease acting on the glutamatergic system by blocking NMDA glutamate receptors. The drugs are primary amines possessing neither prominent UV absorption, nor fluorescence properties nor even electroactive groups. Sample pretreatment with derivatization techniques coupled with GC, HPLC, CE are frequently used for enhancing sensitivity [1].

A new high-throughput liquid chromatography coupled with mass spectrometry (LC/MS/MS) direct method (with derivatization) for quantification of memantine in pharmaceutical formulations was elaborated and validated.

EXPERIMENTAL METHODS

The detection of memantive was in MRM mode using an ion trap mass spectrometer equipped with an atmospheric pressure electrospray ionisation ion source (ESI), positive mode. The analyte was separated on a reversed phase column (Zorbax SB-C18, 100 mm x 3.0 mm I.D., 3.5 μm) under isocratic conditions using a mobile phase of a 55:45 (v/v) mixture of methanol and 0.1% formic acid in water. The flow rate was 1 ml/min at the column temperature 45 ˚C.

In preparing for this study, a series of calibration solutions containing the appropriate standards or spiked placebo were made by adequate dilutions of concentrated stock solutions. The typical concentration range of these calibration solutions was from 0.1 to 1.1μg/ml that covered the 20-120% range of concentrations in the dissolution study, after a 1:10 dilution.

Statistical test

To evaluate the validation of a method there are used the following statistical test: Cochran test, Fisher test and Student test [2,3]. The Cochran test estimates the variance in the group divided by sum of variances of the entire group. If $C_{calc} < C_{theoretical}$ the test is true, i.e. the variance are homogeneous. This test was applied to evaluate the linearity, the accuracy, and the reliability of the method. The Fisher test is mainly used for variance comparison. The ratio of two variance is compared with the $F_{theoretical}$ value. If $F_{cal.} < F_{theoretical}$ the difference is not significant the two variances are coherent. This test is performed to control the least square regression (linearity): the slope must be significantly different to 0 ($F_{cal.} > F_{theoretical}$) and the linearity adjustments must be non-significant ($F_{cal.} < F_{theoretical}$). It is also applied to evaluate the validity of average recovery (accuracy) [3].

RESULTS AND DISCUTIONS

The ion transition monitored was m/z 180 → m/z 136, corresponding to a loss of ammonia from the protonated pseudomolecular ion of memantine (Fig 1).

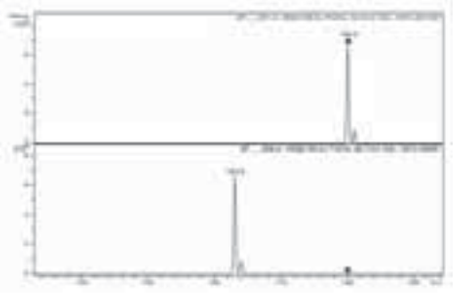

Figure 1: Mass spectra of memantine; upper- full scan spectra, lower- MS/MS spectra

In the chromatographic conditions, the retention time of memantine was 1.80 minutes (Fig. 2).

Linearity. The statistical evaluation of linearity study is presented in Table I. The obtained statistical parameters demonstrate that the method has a good linearity over the considered concentration range.
Accuracy. The study of accuracy was realized for the reconstituted dosage forms, on 5 concentrations (the same concentrations used in linearity study). The analyses were repeated in 3 days.

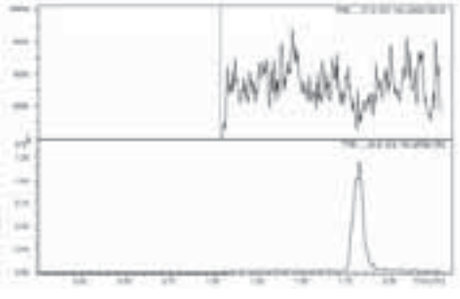

Figure 2: Typical chromatograms for a blank (upper trace) and a sample with memantine (lower trace)

Table 1: Linearity

Tests	Standard sample (S.R.)	Spiked placebo sample	Theoretical values
Curve regression equation	y =7116853x + 113166	y = 6969766.x - 150971	
Correlation Coefficient	0.998	0.992	
Cochran Test - homogeneity of variance (Ccalc)	0.434	0.411	Ct (0.05; 5; 2) = 0.68
Fisher Test - validity of regression (Fcalc)	-2.255	-0.878	Ft (0.05; 3; 10) = 3.71
Fisher Test - significant slope (Fcalc)	3389.353	2293.531	Ft (0.05; 1; 13) = 4.67
Student Test - comparative test of intercept with	3.498	1.403	t(0,05; 13) = 2.160
Student Test - comparative test of slope (tcalc)	0.774		t(0,05; 26) = 2.056
Student Test - comparative test of intercept (tcalc)	-1.159		t(0,05; 26) = 2.056

Table 2: Accuracy

Test	Calculated values	Theoretical values
Cochran Test - comparative test of the homogeneity of variance	Ccalc = 0.392	C(0,05;5,2) = 0.68; nj = 3
Fisher test - validity of average recovery	Ccalc = 3.152	F(0,05;4;10) = 3.48
Confidante interval	94.41 - 102.77	t (0,05; 14) = 2.145

Accuracy. The study of accuracy was realized for the reconstituted dosage forms, on 5 concentrations (the same concentrations used in linearity study). The analyses were repeated in 3 days.

Reliability was appreciated by calculating the intra-day and inter-day variation at 100% theoretical concentration in 6 replicates. The results are shown in table III.

Table 3: Reliability

Test		Value
Cochran test - comparative test of the homogeneity of variances intra-group	Ct (0.05; 5; 2) = 0.68	0.801
Intra - day precision	CVr	5.63%
Inter -day precision	CVR	7.32%

Application. The results obtain to assay tables dosage forms are presented in table IV.

Table 4: Tablet dosage forms assay

Theoretical content (mg)	Results (mg)	Recovery (%)	SD (n=3)	CV (n=3)
10	10.393	103.93%	0.406	3.9%

CONCLUSIONS

In conclusion, the elaborated LC/MS/MS method, more rapid than the other similar methods, can be useful for the rapid quantification of memantine in pharmaceutical dosage forms.

References

1. Cui Shuangjin, Feng Fang, Liu Han, Ma Ming, New method for high-performance liquid chromatographic determination of amantadine and its analogues in rat plasma, *Journal of Pharmaceutical and Biomedical Analysis*, 2007, 44, 1100–1105
2. Piet van Zoonen, Ronald Hoogerbrugge, Steven M. Gort, Henk J. van de Wiel, Henk A. van t Klooster, *Trends in Analytical Chemistry*, 1999, 18 (9-10), 584-593.
3. Roger Wood, *How to validate analytical methods*, Trends in Analytical Chemistry, 1999, 18 (9-10), 624-632.

PO038

Use of isothermal microcalorimetry for evaluation of methionine stability

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INTRODUCTION

The potential range of applications of isothermal microcalorimetry in the pharmaceutical sciences is extremely large, but it has not been widely used, possibly due to the inherent lack of specificity, instrument costs, advances in other techniques and regulatory considerations. The greatest promise for microcalorimetry lies in measurements of very complex systems where a single specific technique would not be applicable [1]. The use of microcalorimetry in stability testing offers much potential, but there is also a need to balance good experimental design with careful interpretation. Investigation of physical and chemical stability of phar-

maceuticals by this technique can therefore be of great importance. Many drugs have been subjected to microcalorimetric investigation, including ascorbic acid, enalapril and perindopril [2-4].

Oxidation is one of the most common decomposition mechanisms studied for drug compounds, where oxidation reactions usually involve complex pathways with multiple intermediates and products [5]. The mechanisms behind these reactions are therefore often poorly understood, making isothermal microcalorimetry a suitable analytical tool for studying these complex processes.

The objective of our study was to evaluate the microcalorimetric technique for monitoring oxidation reactions of amino acid methionine in aqueous solution. The heat flow responses were investigated by testing the impact of an oxidant on the decomposition of selected compound. The results were then compared to those obtained by an HPLC assay that measures the amount of the unchanged compound remaining in the solution.

MATERIALS AND METHODS

L-methionine with purity greater than 99% was obtained from Sigma (USA). Powder was dissolved in water to give the concentrations of 0.5 g/L. The final sample solutions containing 0.3, 0.03 and 0.003% hydrogen peroxide were obtained by adding 30% H_2O_2 to the prepared compound solutions.

The microcalorimeter used in these studies was a MicroDSC III (Setaram, France) operating in the isothermal mode at 25°C. Hastelloy closed batch vessels were used. An aliquot of 850 ml of sample solution was loaded into the sample vessel immediately after solution preparation and the reference vessel was filled with water (850 μl). A 5 minute equilibration time was allowed before data were collected using the built in Setsoft software package. In all experiments the heat flow signal was recorded for 20 hours.

The sample solutions prepared for the microcalorimetric measurements were analyzed with HPLC immediately after preparation to obtain a 100% content value for methionine and directly after the termination of microcalorimetric assay. The HPLC analyses were carried out using a 1100 series HPLC (Agilent, USA). The used HPLC column was Synergy Hydro (250 x 4.6 mm, 4μm) with guard column (4 x 3mm) supplied by Phenomenex (USA). The mobile phase was 0.01% phosphoric acid at flow rate of 1 ml/min and UV detection was set at 215 nm.

RESULTS AND DISCUSSION

The microcalorimetric outputs of methionine under oxidative conditions using hydrogen peroxide at different concentrations are presented in Figure 1. The curves are showing a high start heat flow for all solutions containing H_2O_2. This heat flow then decreases in accordance with concentration of H_2O_2 showing steeper curves in the case of higher oxidant concentration. The microcalorimetric outputs indicates that the rate of methionine decomposition increases with the concentration of oxidant. Chromatographic analyses of methionine after microcalorimetric measurements confirmed rapid oxidation in the presence of hydrogen peroxide. Obtained results for the degradated methionine were as follows: 100%, 97%, 24% and 0.1% for 0.3%, 0.03%, 0.003% H_2O_2 solution and water solution, respectively.

Thus, good agreement was obtained between the profiles of heat flow curves and data obtained by HPLC, which is also in accordance with similar experiments performed on ascorbic acid [4].

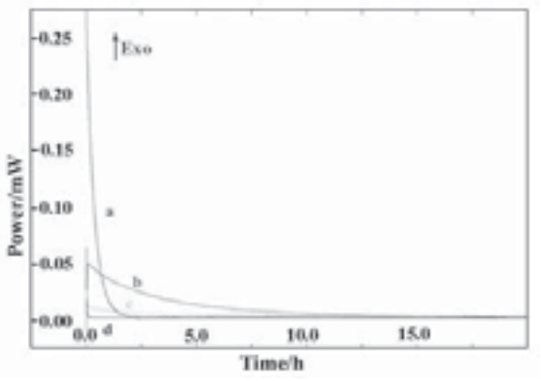

Figure 1: Power-time curves for the oxidation of methionine in (a) 0.3% H2O2 solution; (b) 0.03% H2O2 solution; (c) 0.003% H2O2 solution; (d) water solution.

CONCLUSION

MicroDSC III in isothermal mode has been successfully applied for (in)stability prediction of methionine in solutions. A good agreement between the profiles of heat flow curves obtained by isothermal microcalorimetry and quantitative measurements performed by HPLC has been namely demonstrated in our experiments.

References

1. Koenigbauer M.J.: Pharm. Res. 11: 777-783 (1994).
2. Simoncic Z., Roskar R., Gartner A, Kogej K., Kmetec V.: Int. J. Pharm. 356: 200-205 (2008).
3. Simoncic Z., Zupancic P., Roskar R., Gartner A, Kogej K., Kmetec V.: Int. J.Pharm. 342: 145-151 (2007).
4. Roskar R., Kmetec V.: Eur. J. Pharm. Sci. 25: S180-182, suppl. 1 (2005).
5. Waterman K.C., Adami R.C., Alsante K.M., Landis M.S., Lombardo F., Roberts C.J. : Pharm. Dev. Technol. 7: 1-32 (2002).

PO039

Hi-6 dimethanesulphonate: purity and stability investigation

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INTRODUCTION

Highly toxic organophosphorus-type (OP) chemical warfare agents (nerve agents) and OP pesticides may be used by terrorists and during military conflicts emphasizing the necessity for the development of effective medical countermeasures. The prospective oxime HI-6 dichloride (1-2-hydroxyiminomethyl-1-pyridino-3-(4-carbamoyl-1-pyridino -2-oxapropane dichloride) has been evaluated as an oxime alternative to pralidoxime in the treatment of organophosphorus (OP) poisoning. Another HI-6 salt, HI-6 dimethanesulphonate (HI-6 DMS) appears extremely safe and effective against nerve agents and may be suitable for human use. In this study, we developed an HPLC analytical method to study HI-6 DMS purity and stability.

EXPERIMENTAL METHODS

HPLC analytical method with ultraviolet (UV) detection was developed. The separation was performed on a Atlantis HILIC silica (150 mm x 4.6 mm, 3mum) column. As mobile phase, a mixture of ammonium formiate buffer pH 2, 10 mM, ACN and water (10:45 : 45 v/v/v) was used. Detection is carried out using a UV-vis detector at 254nm. The goal was to well separate isonicotinamide, pyridine 2 aldoxime and HI-6 DMS. On the other hand, Hi-6 DMS was subjected to stress alkaline, acidic, oxidative degradation.

RESULTS AND DISCUSSION

The method was found to be specific, sensitive, precise and linear over a concentration range of 2.50-100 microg/mL and accurate. Accelerated stress testing showed degradation products, which were well separated from the parent compound, confirming its stability-indicating capacity. Hi6 DMS was found to be stable under alkaline and acidic conditions. Nevertheless, the amount of INA and P2A increased under oxidative conditions.

CONCLUSION

HI6 DMS in water is more stable at low temperature; degradation is more pronounced when temperature increase.

Experiments carried out with different pH solutions lead to the conclusion that HI6 DMS is more stable in acid solutions ; indeed the more the pH of the solution increases the more HI6 DMS degrades. The main degradation products detected are INA and P2A but another unknown peak has been observed. After two days under oxydative conditions, the proportion of INA Dimer has increased and a peak of P2A has been detected.

REFERENCES

1. Pharmacopée Européenne 5ème ed. (2005), Conseil de l'Europe, Strasbourg, France.
2. Lundy PM, Hill I, Lecavalier P, Hamilton MG, Vair C, Davidson C, Weatherby KL, Berger BJ. The pharmacokinetics and pharmacodynamics of two HI-6 salts in swine and efficacy in the treatment of GF and soman poisoning. 2005 Mar 30;208(3):399-409.

PO040

Influence of temperature on stability of celecoxib-loaded microemulsions

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INTRODUCTION

Microemulsions, dispersions of oil and water, stabilised with a surface active film composed of surfactant and cosurfactant, are potential drug carrier systems for oral, topical, and parenteral administration. Due to very low interfacial tension between the oil and water phase they form spontaneously and are thermodynamically stable. Low interfacial tension is further related to a wide range of microemulsion structures possible. In general, thay can be separated into 3 types: water-in-oil, bicontinuous, and oil-in-water microemulisons (1). Microemulsions are stable, however they are not an inert vehicle. Addition of the active ingredient in the pharmaceutical microemulsions may therefore affect its phase behavitor and thus the microstructure and stabilty of the system (2).

The aim of our work was therefore to evaluate the microstructure stability of celecoxib loaded micoemulisons and chemical stability of celecoxib during 3 months storage at 40°C.

EXPERIMENTAL METHODS

Composition of microemusions:

- Lipophilic phase: Mygliol 812® (Hüls, Germany)
- Surfactant: Labrasol® (Gattefosse, France)
- Cosurfactant: Plurol oleique® (Gattefosse, France)
- Hydrophilic phase: double distilled water
- Model drug: celecoxib (Lek Pharmaceuticals d.d.)

Preparation of microemulsions:

The surfactant and cosurfactant were blended in a 4:1 mass ratio to give a surfactant mixture. A stock solution was prepared comprising 88 wt.% of this mixture and 12 wt.% of Mygliol®. The appropriate amount of water was then added to obtain the desired microemulsion composition (Table 1). Drug loaded microemulsions were prepared by adding celecoxib to already prepared systems in 1 % concentration.

Stability testing:

Tightly closed glass flasks with microemulsion samples were stored light-protected at 40°C for 90 days. At different time intervals (0, 45 and 90 days) they were evaluated for their organoleptic properties, homogenicity, transparency, conductivity properties (Iskra conductivity meter MA 5964, Slovenia), viscosity (Hoeppler viscometer) and drug content. All measurements were performed at 20 ± 0,5°C. Chemical stability of celecoxib was deterimined by HPLC analysis (Agilent 1100, Agilent, USA).

Table 1: Composition of the characterised samples

Sample	Water [%]	Miglyol [%]	Emulsifier [%]
1	0,00	12,00	88,00
2	4,76	11,43	83,81
3	11,11	10,67	78,22
4	16,70	10,00	73,30
5	21,60	9,41	68,99
6	27,27	8,73	64,00
7	31,03	8,28	60,69
8	36,51	7,62	55,87
9	41,18	7,06	51,76
10	45,21	6,58	48,21
11	50,00	6,00	44,00
12	55,55	5,34	39,11

RESULTS AND DISCUSSION

After 45 and 90 days storage at 40°C all samples (except Sample 1) remain transparent and no phase separation could be observed. However, after 90 days they become slightly darker, probably due to the temperature-induced oxidation of Plurol Oleique®. Viscosity (Fig. 1) and conductivity (Fig. 2) data confirmed that microemulisons suffered no considerable structure changes during the first 45 days, whereas some difference in viscosity and conductivity curves could be observed after 90 days storage at 40 °C.

The viscosity of systems increases with increase in water phase volume fraction (Φ) from 0 to 11 wt.% which is in agreement with previous findings (3). With further increase in Φ up to 55 wt.% a decrease in the viscosity is observed. However, the decrees in viscosity is less pronounced when Φ is between 27 and 45 wt.%. This could be due to the clustering of the droplets at the percolation threshold which typically leads to an increase in viscosity and conductivity (4). In agreement with viscosity measurements the conductivity profile reveals three different regions. In the region with low water content (Φ < 27 wt.%) the conductivity of selected samples is low. In the middle region (27 wt.% < Φ < 45 wt.%) the conductivity increases linearly (k = 7,13) and sharply. With further increase of Φ (Φ > 45 wt. %) the slope of the curve decreases (k = 5,05). Thus the viscosity and conductivity curve probably suggest transformation of system structure from oil continuous (Φ < 25 wt.%), via bicontinuous (27 wt.% < Φ < 45 wt.%), to water continuous (Φ > 47 wt.%).

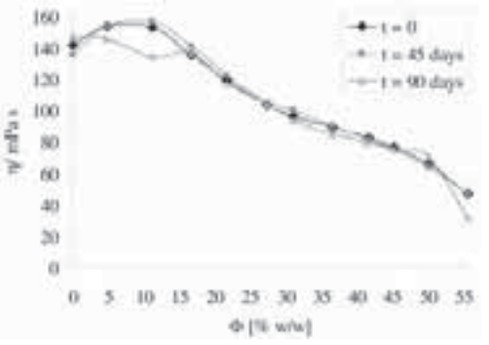

Figure 1: Dynamic viscosity of selected microemulsions as a function of the water ratio after 0, 45 and 90 days storage at 40 °C.

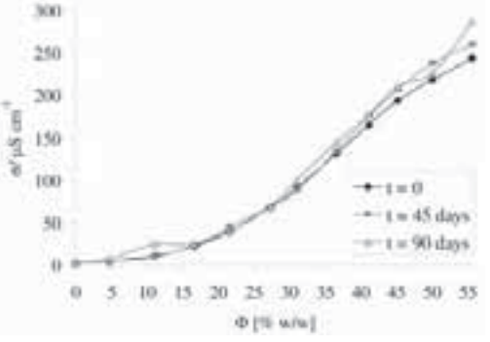

Figure 2: Electrical conductivity of selected microemulsions as a function of the water ratio after 0, 45 and 90 days storage at 40 °C.

After 90 days storage at 40°C the shape of the viscosity curve changed considerably at Φ < 17 wt.% and Φ > 45 wt.%. This probably indicates some changes in the systems microstructure; however, additional tests would be needed to explain precisely the alteration observed. In agreement to these results, the conductivity of the systems with Φ below 17 wt.% increased due to the decrease in viscosity observed after 90 days storage at 40°C. Similarly, at Φ = 50 wt.% the conductance was lower than at time 0 due to higher viscosity measured after 90 days.

Stability of celecoxib in the formulations, which was defined as the retention of at least 90 % of the initial concentration, was also tested. Results of HPLC analysis showed that all microemulsion samples (accept

Sample 12) are considered as stable during 90 days storage at 40°C (Fig. 3).

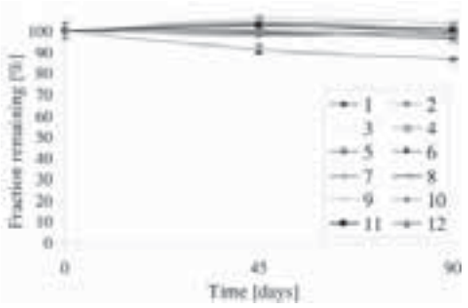

Figure 3: Stability of 1 % celecoxib in microemulsions samples 1-12 stored at 40 C for 90 days.

CONCLUSION

Stability testing of celecoxib-loaded microemulsions revealed that microstructure changes are not very likely to occurre during 45 days storage at 40°C. Considerable changes in viscosity and conductivity of the systems with water phase content below 17 wt.% and above 45 wt.% were observed after 90 days, which probably indicate alteration of their microstructure. However, systems consisting of 17 to 45 wt. % water did not suffer any changes and remain stable throughout the stability test. The stability of celecoxib was adequate when water phase content was kept below 55 wt%.

Reference

1. Tenjarla S. Crit. Rev. Ther. Drug. Carrier Syst. 1999, 16 (5): 461-521.
2. Bagwe et al, Crit. Rev. Ther. Drug Carrier Syst. 2001, 18 (1): 77-140.
3. Bennet at al. In: Robb, I.D. (Ed.), Microemulsions. Plenum Press, New York, pp. 65–84.82.
4. Podlogar et al. Int. J. Pharm. 2004, 276: 115–128.

PO041

Tautomeric polymorphism in omeprazole isomers: Does it affect compatibility with mannitol?

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INTRODUCTION

Omeprazole is a is a blockbuster anti-ulcer drug. However, its poor solubility and physiochemical instability results in formulation and bioavailability issues. Since S-omeprazole shows more predictable bioavailability and excipients have been known to interact with active pharmaceutical ingredients to produce altered bioavailability, it was decided to investigate the compatibility of omeprazole sodium isomers with mannitol, the major excipient in omeprazole formulations using differential scanning calorimetry (DSC), attenuated total reflectance infrared (ATR-IR) spectroscopy in a powder mixture and localized thermal analysis (LTA) from a drug disk.

EXPERIMENTAL

Physical mixtures of S- and R-omeprazole and mannitol were prepared in different weight ratios (20:80; 50:50 and 80:20) S- and R-omeprazole-mannitol disks were prepared by placing approximately 200 mg of powder sample into a die and applying pressure of ~7 tonnes for 5 minutes for use in ATR-IR and micro thermal analysis experiments. ATR-IR powder samples were prepared as smear mounts, where approximately 0.5 g of each sample was the mixed with water and smeared directly onto the crystal.

ATR-IR spectra of the compressed omeprazole-mannitol mixtures were measured directly, with themannitol disks placed in intimate contact with the ZnSe crystal and spectra were recorded.

The DSC measurements were conducted using a DSC 2920 instrument (TA Instruments, New Castle, DE).

(μTA) was conducted using a 2990 Micro-Thermal Analyser (TA Instruments, New Castle, DE) with a thermal probe. Scans were performed at 30°C, with a scan rate of 1 Hz, in triplicate. Additionally, localized thermal analysis (LTA), at a heating rate of 10°C s^{-1} for up to 300°C, was applied and reproducibility confirmed.

ATR-IR spectra were collected using a Nicolet "Nexus" FTIR spectrometer with ZnSe prism (4000–650 cm^{-1}).

RESULTS AND DISCUSSION

Addition of mannitol to S- and / or R-omeprazole leads to the decrease in the crystalline nature of the resulting mixtures. In the case of the S-omeprazole-mannitol mixture, a decrease in the melting temperature for mannitol is evident. In addition, mixing of R-omeprazole and mannitol resulted in the decrease in melting temperatures for mannitol and broadening the melting peaks due to the interaction of two different crystalline structures and melting of omeprazole (Figure 1)

LTA was used to visualize the spatial distribution in compressed mixtures, to display images of the sample's topography and to perform a microthermal analysis. Topographical images (Figure 2) revealed better compression behaviour of S-omeprazole-mannitol mixture resulting in smoother surface compared to that of the R-omeprazole-mannitol mixture. It is apparent from the surface topographical images that the "peaks" correspond to a significant extent with a particular type of large `aggregate morphology. It is not unreasonable to suggest that this morphology is related to one of the polymorphic forms of omeprazole. Great variations in the depth of the peaks probably arise from a varying con-

tact areas and packing density of material under the probe, but could be also due to varying ratios of amorphous and different crystalline material. Thus LTA on a mixture of omeprazole-mannitol has identified the darker and the lighter regions as different polymorphs.

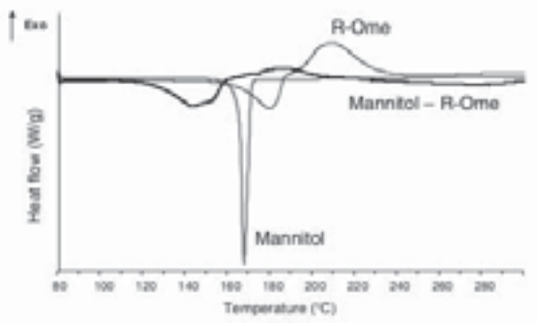

Figure 1: DSC thermograms of and pure R-omeprazole (R-Ome) and mannitol and R-omeprazole-mannitol(Mannitol-R-Ome) mixture (50:50), (heating rate of 0.5 C min-1).

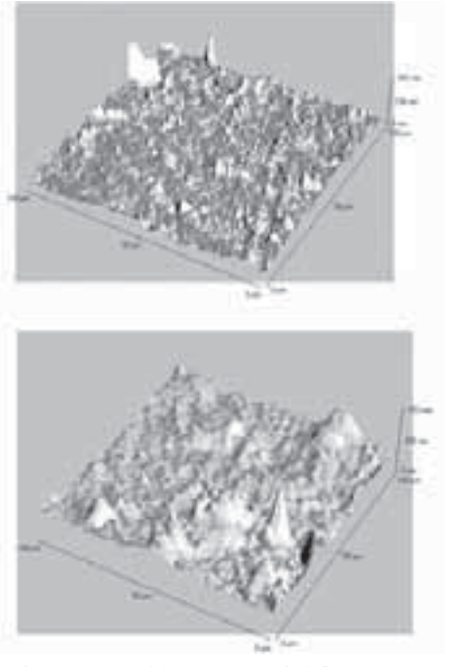

Figure 2: Surface topographical images of a) S-omeprazole - mannitol and b) R-omeprazole - mannitol compressed disks.

Generally, such behaviour was not observed with the DSC method, where a peak corresponding to a statistical average of the varying crystalline and amorphous content of the material was absent. In the case of S-omeprazole, no significant peaks were recorded supporting previous findings that esomeprazole is mostly amorphous [1].

The ATR-IR spectra of the S- and R-omeprazole-mannitol powder mixtures did not show a significant difference. However ATR-IR spectra obtained from the drug disk have a profound difference in the N-H vibration region. The R-omeprazole-mannitol mixture has two distinct peaks due to amino (N-H) and imino (=N-H) stretching frequency, at 3425 and 3318 cm-1 respectively, while S-omeprazole-mannitol mixture showed only a peak due to amino N-H stretching vibration and a small shoulder peak from the imino stretching vibrations (Figure 3). It was recently confirmed that crystalline omeprazole exists as solid solutions of two tautomers in a continuous composition range [2]. Therefore S-omeprazole consists of predominantly only one tautomeric isomer.

Figure 3: ATR-IR spectra of S- and R-Omeprazole- mannitol mixtures (70 : 30 w/w) from compressed disks.

CONCLUSION

Results from this study may be used to explain the unpredictable bioavailability of R-omeprazole as compared to S-omeprazole. The omeprazole sodium isomers demonstrated different thermal profile and degree of crytallinity. Because of the potential impact bioavailability and thus therapeutic efficacy, this should definitely be assessed in the preformulation stages in the development of omeprazole dosage forms.

References

1. Markovic N., Agotonovic- Kustrin S., Glass B. and Prestidge C.A., Physical and Thermal Characterisation of Chiral Omeprazole Sodium Salts, J. Pharm. Biomed. Analysis, 42 : 25-31 (2006).
2. Bhatt P.M. and Desiraju G.R., Tautomeric polymorphism in omeprazole , Chem. Commun. 28 : 2057–2059 (2007).

PO042

Utilization of ^{14}N nuclear quadrupole resonance for qualitative and quantitative study of polymorphism

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INTRODUCTION

Solid-state research plays an important role in any industry that is involved in the production or processing of solid matter. This is especially true in the pharmaceutical industry, since most of active ingredients exist as polymorphs, solvates or amorphous forms. Precise, accurate and quick differentiation and categorisation of these states is of paramount importance, because they have impact on stability, bioavailability, formulation, quality control and regulatory issues. Nuclear quadrupole resonance (NQR) is similar to NMR with the difference that in NQR the energy levels are determined by the coupling of electric field gradient (EFG) and nuclear quadrupole moment. (the quadrupole interaction). Transitions between these levels are stimulated in NQR and in NMR by the action of radiofrequncy (rf) field. Chemical bonds are important in establishing the EFG. When certain conditions are met, NQR can easily give precise and unique information on the potential deviations from the expected electronic distribution in the crystal lattice due to: polymorphism, phase transitions, crystal water, lattice vibrations, etc. NQR is a contactless, nondestructive, exact-structure sensitive method that generally requires no special sample preparation. Nuclear quadrupole moments are intrinsic properties of nuclei that arise from the spatial distribution of charge in the nuclei (1). Only the nucleus with spin greater than (1/2) can have quadrupole moment. When the electric charge distribution around the nucleus is not symmetric, it gives rise to electric field gradient that interacts with the qudrupolar nucleus to give different quadrupolar energy levels. The field sources are the neighbouring nuclei and all the surrounding electrons. Usually the predominant contribution comes from the electrons forming the chemical bonds of the resonant atom. The pure NQR frequency spectrum is determined entirely by the geometry of the electric charge distribution in the compound's structure lattice and as such is highly sensitive to changes in it. Nitrogen nucleus has a spin of one. It is also ubiquitous in many pharmaceutical active ingredients and excipients as well. Three different active pharmaceutical ingredients (API) have been chosen that were likely to give significant detectable ^{14}N-NQR lines in the frequency spectrum.

EXPERIMENTAL METHODS

Suitable solid state forms of sulfanilamide, piroxicam and nifedipine were prepared according to the instructions found in the literature (2, 3, 4). The samples were scanned for possible NQR frequencies employing double resonance technique that can in appropriate circumstances determine ^{14}N NQR frequency via the coupled neighbouring proton NMR signal change, caused by the artificially induced transient ^{1}H-NMR/^{14}N-NQR level crossing. The potential NQR frequencies have been scanned in details by pulse NQR spectrometer. SpinCore Pulse Blaster and Imtec analog to digital converter, both as computer plug-in cards, were used for running pulse programs and for acquisition and processing of signals, respectively. They were controlled by the SeveNMR computer software developed at Institute Jožef Stefan, Ljubljana. Analog phase sensitive signal detection (quadrature) was done by the prototype unit Double conversion NMR spectrometer S.E.A. 89, which requires stable rf frequency reference and clock synchronisation that was supplied by Agilent 33250A Waveform Generator. The low power rf excitation pulses were amplified by Tomco rf Pulse Amplifier. The amplified high power pulses were fed into a standard home-made impedance-matching circuit with a solenoidal sample coil of about 5 cm^3 volume. Multipulse spin-lock spin echo sequence has been employed for efficient signal acquisition. The refocused echoes between the p-pulses were accumulated on-line. Programs written in Mathematica 6 were used for the treatment of the acquired data. Frequency spectra were produced and the peak heights measured and plotted versus the chosen variables. The efficiency of the signal detection was rationalized by choosing of sample dependent optimal length of the and the number of p-pulses in a single sequence. The detected signal represents the precessing nuclear magnetization refocused in the rotating frame, determined by the reference frequency. In our case the excitation frequency and the reference frequency were the same. IR spectroscopy, hot stage microscopy and DSC were employed as the reference methods.

RESULTS AND DISCUSSION

Sulfanilamide

Three polymorphic forms of sulfanilamide were prepared (2). They were examined by DSC, IR and thermal microscopy to confirm the differences among them. NQR frequencies for sulfanilamide were found from a previous NQR study (5). The resonance frequencies have been scanned with the NQR spectrometer and as it has been expected four strong lines for the β form have been identified. However, no resonance lines in the spectra of the other two polymorphs were found so far in spite of having the double resonance data. Anyhow, the detection and optimization of experimental parameters of ^{14}N NQR resonances in β-sulfanilamide gave us useful orientational parameters for our further ^{14}N-NQR measurements in pharmaceutical compounds. NQR spectra of β-sulfanilamide could be used for distinguishing it from the other two polymorphs, since we did not observe any significant spectral lines at those monitored frequencies.

Piroxicam

Two polymorphic and a monohydric form (4) have been examined. As before, they have been examined with reference methods to confirm differences among different forms. Double resonance technique gave approximate resonance frequency positions for form I that were confirmed and more accurately defined with the NQR spectrometer.

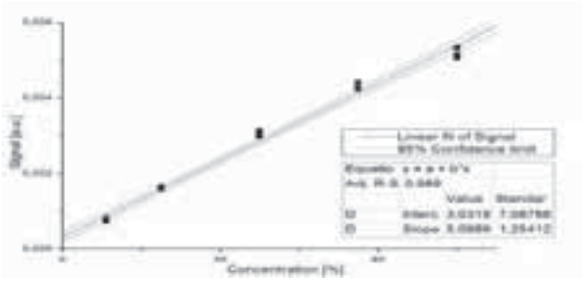

Figure 1: Graph of the piroxicam I ^{14}N-NQR (2.587MHz) signal versus its concentration relationship with 95% confidence limits.

Piroxicam I and monohydric form were mixed at different ratios. ^{14}N-NQR signal intensity versus concentration plot at lower frequency (2.587MHz) shows as expected a distinct linear trend with narrow confidence limits. The same was done for the higher frequency (3.440MHz), where only the free induction decay was observable. There the signal to noise ratio was smaller, what was the reason for more scattered results. This indicates that the lower frequency in piroxicam is more suitable for concentration measurements.

Nifedipine

Double resonance examination of form I revealed two complete sets of resonances at room temperature, belonging to two different ^{14}N atoms in the molecule. They were in the region around 1MHz and 2.5MHz. The higher set was chosen for further examination with the NQR spectrometer. Theoretically, the NQR signal intensity is proportional to the number of resonating nuclei as was shown in the piroxicam study above. The same relationship has been assumed for the nifedipine form I crystals. First examination of the newly prepared amorphous phase at room temperature gave no sign of change in the time interval (a few days) we were observing it. We then proceeded with the process of heating the sample for a defined time interval at a fixed temperature, then cooling it quickly to room temperature and recording the NQR spectra. The time evolution of the recrystallization process of form I exhibits a typical sigmoidal behaviour (Fig. 2). Similar signal dependency on the time of heating was obtained for 65°C, 75°C and 80°C. The time of onset of the crystallization, its rate, and also the seeming plateau observed during measurement were dependent on the temperature of heating.

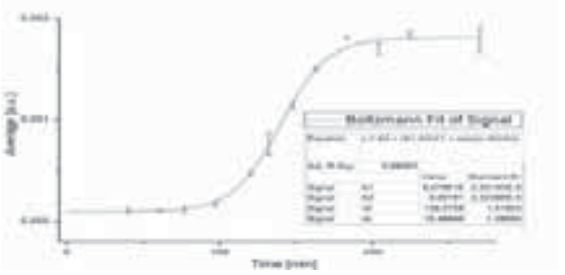

Figure 2: Boltzmann function fit of the nifedipine ^{14}N-NQR (2.590MHz) signal intensity versus time of heating at 70 C temperature.

CONCLUSION

The NQR examination of β-sulphanilamide yielded strong resonance frequencies. At these frequencies the other studied polymorphic species do not resonate. This proves that the NQR method is capable to differentiate one polymorphic form from others, provided that at least one set of resonance frequencies is known, and checked that no other known form resonates at these frequencies.

NQR signal is proportional to the concentration of resonating nuclei, what has been showed with piroxicam. The method is relative and needs a known reference standard to be measured every time the system is fine tuned. It also needs the system parameters to be defined as much as possible. Then reasonably accurate measurements with ^{14}N-NQR are possible.

Amorphous nifedipine recrystallizes at studied temperatures to the thermodynamically stable form I. With raising the temperature of recrystallization we achieve shorter time of onset, higher rate and higher plateau of the recrystallization. This allows for calculation and prediction of the stability of the amorphous phase of nifedipine. It is possible that some intermediary thermodynamically metastable form develops during recrystallization that is in equilibrium with stable form I. Interesting is the plateau, that is reached and that is dependent more on temperature than on time of recrystallization. More research is needed for providing an accurate description of such nifedipine s transitions.

References

1. Nakamura, N. *Nuclear Quadrupole Resonance Spectroscopy Data.* [ed.] H. Chiahara: Springer. 978-3540624288.
2. A. Portieri, R. K. Harris, R. A.Fletton, R. W. Lancaster, T. L. Threlfall., 2004, Magnetic Resonance in Chemistry, 42, 313-320.
3. Yukio Aso, Sumie Yoshioka, Shigeo Kojima, 2001, Thermochimica Acta, 380, 199-204.
4. F. Vrečer, M. Verbinc, A. Meden, 2003, Int. J. Pharm, 256, str. 3-15.
5. Robert Blinc, Janez Seliger, Aleksander Zidanšek. Veselko Žagar, Fani Milia, Hector Robert, 2006, Solid State Nuclear Magnetic Resonance, 30, 61-68.

PO043

Differential scanning calorimetry (DSC) studies of topical liquid crystal drug delivery sytems containing azelaic acid

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INTRODUCTION

Azelaic acid is a saturated dicarboxylic acid, which has a straight chain composed of nine carbon atoms. It is naturally found in rye, barley and wheat but can also be obtained by the reaction of oleic acid and nitric acid [1]. Its therapeutic effect drew attention when it was found that it could be used in the treatment of hyperpigmentory disorders and acne. In vitro and in vivo studies showed that azelaic acid has antimicrobial, antiinflammatory, antitumoral, antiproliferative properties and also is bacteriostatic against some aerobic and anaerobic bacteria [2]. Altough not toxic nor teratogenic to normal skin, azelaic acid shows selective cytotoxicity against melanoma cells and prevents the proliferation of these cells [3]. Azelaic acid is commercially available as cream (%20) and gel (%15) formulations , which are both approved by the FDA. Extended use of these formulations can give rise to mild erythema, stingling and itching. Thus, new drug delivery sytems which contain low doses of the active ingredient and release the drug in a controlled manner is needed.

Liquid crystals are promising systems for this purpose. They are transition states, possessing both the crystalline characteristics of solids and flow properties of liquids [4]. They are composed of oil, surfactant, water, and generally cosurfactant. They have the advantage of being easily produced, increasing solubility of poorly water-soluble compounds, delivering fragile substances, and controlling release of drugs [5,6].

The aim of the study was to characterize lyotropic liquid crystal sytems for topical application of azelaic acid. DSC measurements were performed to investigate the thermotropic behavior of the formulations.

EXPERIMENTAL METHODS

• Preparation of Formulations

Binary and ternary sytems of liquid paraffin, non-ionic surfactants (Brij 72 and Brij 721P) and water were studied. The samples were prepared by heating liquid paraffin and surfactants to 70 ºC in a water bath. Then, distilled water at 70 ºC was added during constant stirring at 600 rpm. The formulations were stirred until they reached room temperature. The systems were kept at room temperature for 24 hours to establish equilibrium prior to analysis. The phase boundaries were established using polarized light microscopy (Leica DM EP, Germany) at a maximum magnification of 63x.

• Release Studies

In vitro release studies were performed using vertical Franz diffusion cells. Cellulose acetate membranes with MWCO of 12000 – 14000 Da were used. The release medium was composed of pH 7.4 phosphate buffer:ethanol (50:50).

• HPLC Analysis

Samples were withdrawn at determined time intervals and quantitative analysis was performed using a RP-HPLC. The mobile phase was methanol:water (3:2) that contained 10 mM ammonium acetate (pH 5.0 ± 1.0). The flow rate was 1 mL/min. A Hypersil ODS (C18) column with 25x4.6mm i.d. was used during the experiment. Azelaic acid was detected at 210 nm.

• DSC Analysis

In order to characterize the formulations, DSC analyses were performed. The measurements were carried out with a DSC Q100 (TA Instruments, USA). Samples (5–10 mg) were weighed in aluminum hermetic pans and immediately sealed by a press. The reference was an empty pan. The samples were heated at a heating rate of 10°C.min^{-1} from 20 to 80 °C. The heat flow was measured as a function of the temperature.

RESULTS AND DISCUSSION

• Ternary Phase Diagram

Figure 1: Ternary phase diagram for Brij 72:Brij 721P (1:3)/ liquid paraffin/ water ternary system (A = association colloid region, B = emulsion region, C= lamellar liquid crystal, Lα, region) [7].

Ternary phase diagrams with different ratios of Brij 72:Brij 721P (1:3, 2:3 and 3:2) were constructed. Investigations on macroscopic and microscopic properties showed that the optimum ratio for Brij 72:Brij 721P was 1:3. Figure 1 shows the ternary phase diagram for surfactant/oil/water combination where the Brij 72:Brij 721P ratio was 1:3.

• In Vitro Release Studies

For release studies, four formulations were chosen from the A, B and C regions of the ternary phase diagram. The release profile of azelaic acid from the association colloid, lamellar liquid crystal and o/w emulsion was evaluated [7]. The maximum rate and amount of release for azelaic acid was found for the ternary lyotropic liquid crystal formulation,

whereas the minimum was found for the association colloid formulation. The release rates were found as 129.91 ± 0.34, 119.84 ± 0.20, 106.46 ± 0.39, 97.76 ± 0.50 $\mu g.cm^{-2}.h^{-1/2}$, respectively, for the ternary and binary lyotropic liquid crystals, o/w emulsion and association colloid. The release profile of azelaic acid from these binary and ternary systems could be fitted to Higuchi kinetics [7].

• DSC Analysis

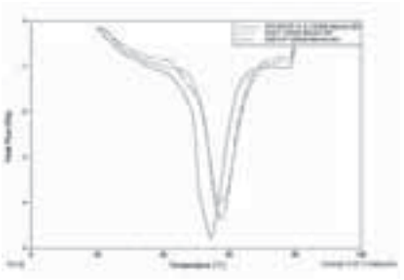

Figure 2: DSC thermograms for Brij 72, Brij 721P and 1:3 mixture of Brij 72:Brij 721P.

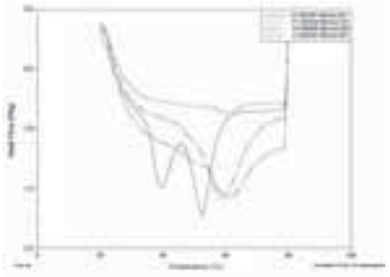

Figure 3: DSC thermograms for ternary and binary lyotropic liquid crystals, o/w emulsion and association colloid formulations without azelaic acid.

DSC analysis showed that Brij 72, Brij 721P and their mixture (Brij 72: Brij 721P (1:3)) had nearly the same melting point, which was found as 57.38 °C, 57.47 °C, 54.34 °C, respectively. As Brij 721P is polyethylene (21) stearyl ether and Brij 72 is polyoxyethylene (2) stearyl ether, they have a similar structure so their melting points are very similar. Their mixture showed a small shift, which indicates that these two surfactants interact with each other. Among the formulations, binary and ternary lamellar liquid crystals and o/w emulsion showed similar peaks, the intensity of which varied with concentration of surfactant mixture. On the other hand, surfactant/water (lyotropic liquid crystal) and oil/surfactant (association colloid) systems showed different peaks in the thermogram, which may be explained by different packing manner and spatial arrangement of the surfactants mixture in different solvents. This difference in thermogram also can explain the difference between the release rates of these two systems.

CONCLUSION

Lamellar liquid crystal, emulsion and association colloid formulations were prepared and characterized. The DSC thermograms showed that the composition of the vehicle affects the intensity and the location of the peak. Studies are underway for the evaluation of azelaic acid containing formulations by DSC.

ACKNOWLEDGEMENT

The authors are thankful to ORVA Pharmaceutical Company, Istanbul for the generous gift of azelaic acid.

References

1. Akamatsu H. . Arch Dermatol Res. 283: 162 – 166 (1991).
2. Bojar R.A. Disruption of the transmembrane pH gradient- a possible mechanism for the antibacterial action of azelaic acid in Propionibacterium acnes and Staphylococcus epidermidis. J Antimicrob Chemother. 34(3): 321 – 330 (1994).
3. Blount B.W. Roseca: A Common, Yet Commonly Overlooked Condition Am Fam Phys. 66(3): 435 – 439 (2002).
4. Savic S. . Colloid Polym Sci. 283: 439 – 445 (2005).
5. Burducea G. Lyotropic liquid crystals. I. Specific structures Rom Rep Phys. 56(1): 66 – 73 (2004).
6. Burducea G. Lyotropic liquid crystals. II. Structural polymorphism Rom Rep Phys. 56(1): 87 – 93 (2004).
7. Aytekin M., Gürsoy N., Hekimo lu S., Formulation and Evaluation of Topical Liquid Crystal Drug Delivery Systems Containing Azelaic Acid, 35th Annual Meeting & Exposition of the Controlled Release Society, New York, USA, 2008.

PO044

Stability of ascorbyl palmitate in microemulsions with additional antioxidant

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INTRODUCTION

UV irradiation induces free radical formation in the skin, leading to premature aging and cancer. The skin possesses a wide range of coupled antioxidants, but the capacity of this sistems is limited. Supporting the cutaneous defence systems with exogenous antioxidants could thus prevent radical mediated damage in the skin. Ascorbyl palmitate (AP) is an amphiphilic derivative of vitamin C with improved chemical stability. AP is widely used in topical preparations against oxidative changes

of biological components of the skin (1-2% concentration) and as an antioxidant to protect lipophilic ingredients in formulations in concentration range 0,05-0,1% (1).

Microemulsions (ME) are clear, thermodinamic stable, isotropic mixtures of immiscible liquids (oil and water) which are stabilised with the interfacial film of surfactant and/or cosurfactant (2). They are especially convenient for cutaneous application because they may act as penetration enhancers, have excellent solubilisation capacity of drugs and may increase the chemical stability of some active compounds.

Our earlier stability studies on AP in ME showed that its long-term stability in ME is still not adequate. In this work the incorporation of additional antioxidant (oxime) into ME for topical application was studied to improve chemical stability of AP.

EXPERIMENTAL METHODS

Tested ME of w/o and o/w type were composed of the medium chain triglyceride Miglyol 812® (Sasol, Germany) as lipophilic phase, caprylocaproyl macrogolglycerides Labrasol® (Gattefosse, France) as surfactant, polyglyceryl-6 dioleate Plurol oleique® (Gattefosse, France) as cosurfactant and double distilled water as hydrophilic phase (Table 1). AP (purchased from Fluka BioChemica) was incorporated into microemulsions at 1% w/w concentration. Oxime (Figure 1) was synthesized at the Faculty of Pharmacy, Ljubljana, Slovenia and incorporated into microemulsions at basic molar ratio AP:Oxime=10:1. To evaluate the main mechanism of oxime action double distilled water was replaced with 0,01 mM solution of metal ions ($FeSO_4x7H_2O$) in o/w ME. Samples with 0,03 mM solution of EDTA (Merck, Germany) as hydrophilic phase were also prepared for the same purpose.

Table 1: The composition of w/o and o/w microemulsions (w/w%).

Component	w/o	o/w
Miglyol®	24,75	7,43
Labrasol®	47,53	38,02
Plurol oleique®	11,88	9,50
Water	15,84	45,05

AP and oxime were disolved in Labrasol and the other three components were then added. Proportions of ME components were kept constant during the addition of antioxidants. ME were formed spontaneously after gentle hand mixing. Samples were stored in well-closed 25 ml glass flasks at room temperature in the dark.

The amount of nondegraded AP and oxime were determined quantitatively by HPLC analysis at predetermined time intervals during two months of storage. Briefly, the stacionary phase was 120x40 mm id. column packed with 5 μm Eurosphere C18. The mobile phase consisted of methanol, acetonitrile and 0,02 M phosphate buffer pH 3,5 at the ratio 75:10:15 (v/v). The flow rate was 1,5 ml/min. The UV detector was set at the wavelength of 254 nm. The injection volume was 50 μl.

RESULTS AND DISCUSSION

Previous studies have determined large amount of AP degradation in w/o and o/w ME. However, AP was more stable in w/o ME (2). This is due to the fact that the cyclic ring of AP, which is sensitive to oxidation, is located in the internal aqueous phase and the w/o interface act as a barrier for the diffusion of oxygen to the internal aqueous phase (3). On the contrary, the addition of oxime in molar ratio AP:Oxime=10:1 improved stability of AP in o/w ME (Table 2), but not in w/o ME (data not shown). The primary scope in oxime synthesis was the chemical structure analogy to the AP molecule in order to get similar distribution in ME. The obtained higher stability of AP in the presence of oxime in o/w ME can be explained with different partition patterns of AP and oxime in ME. AP as amphiphilic molecule is soluble in lipophilic phase and surfactant/cosurfactant mixture but not in hydrophilic phase. Because of a smaller volume of lipophilic phase in the case of o/w ME the local concentration of AP is higher in inner phase. Presuming the similar oxime structure, we can also anticipate its higher local concentration in inner phase of o/w ME and consequently higher stability of AP.

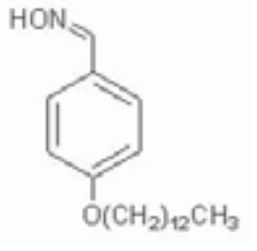

Figure 1: Chemical structure of oxime.

The experiment with higher molar ratios of oxime (10:2 and 10:4) revealed the same result. No effect was observed in w/o ME, whereas AP stability was highly increased in o/w ME. An optimal molar ratio is 10:2, since higher molar ratio gave similar results (Table 2).

Table 2: Nondegraded AP (percentage) in o/w ME with different incorporated components after two months of storage.

Sample (O/W)	% AP
AP	27,8 ± 2,9
AP+Oxime (10:1)	46,9 ± 3,6
AP+Oxime (10:2)	54,3 ± 6,2
AP+Oxime (10:4)	52,9 ± 1,8
AP+Oxime+Fe^{2+}	1,5 ± 0,1
AP+EDTA+Fe^{2+}	8,7 ± 2,6
AP+Oxime+Fe^{2+}+EDTA	19,9 ± 8,8
AP+EDTA	41,0 ± 3,7

There are two possible mechanisms for increasing the AP stability in the presence of oxime – it can either chelate metal ions present in formulation or directly react with free radicals. Our preliminary experiments could not confirm oxime acting as a chelating agent. When EDTA solution alone was incorporated in the system, the percentage of nondegraded AP was considerably higher comparing with the system where metal ions were additionally added (41,0% compared to 8,7%). We can therefore assume that EDTA is not capable to chelate supplemental metal ions present in the system. When oxime was additionally added to this system, the percentage of nondegraded AP increased from 8,7% to 19,9%. This allows us to anticipate that oxime could chelate the residual metal ions. However, our preliminary results of AP stability in the presence of excess metal ions and oxime did not confirm this, since al-

most all AP was degraded after two months of storage. It is possible that the concentration of oxime was to low; therefore further studies with higher oxime molar ratios will be done (Table 2). Furthermore, antioxidative property of oxime should also be considered.

CONCLUSION

- incorporation of oxime improved stability of AP in o/w ME
- oxime is most effective in molar ratio 10:2
- in the presence of metal ions synergistic effect of oxime and EDTA on AP stability was observed

References

1. Lawrence MJ, Ress GD. Adv Drug Del Rev 2000; 45: 89-121.
2. Špiclin P, Gašperlin M, Kmetec V. Int J Pharm 2001; 222: 271-279.
3. Gallarate M, Carlotti ME, Trotta M, Bovo S. Int. J. Pharm 1999; 188: 233-241.

PO045

Effect of rubber stopper selection on development of stabile lyophilized product

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INTRODUCTION

During laboratory development of lyophilized product several factors are to be considered. Besides setting a rational process that results in acceptable and stable product the selection of suitable container closure system must be evaluated. Residual moisture content of product often correlates with higher degradation and lower product stability. The source of moisture could be autoclaved rubber stoppers, sometimes in direct contact with dry lyophilized product. We showed that additionally dried rubber stoppers would result in sufficiently low moisture content and thus be suitable for our product. In addition, high pH of the product was necessary to assure proper stability. Because of very high pH, effect of CO_2 permeability of rubber stoppers was also evaluated. It was shown that lowering pH of lyophilized product due to CO_2 permeability through the stopper has a pronounced negative effect on product stability. Considering the influence of both factors on stability (residual moisture content and CO_2 permeability), a suitable rubber stopper was selected for our product.

MATERILAS AND METHODS

We developed a lyophilized powder for infusion with active substance consisting of a synthetic form of prostaciclyn. This very unstable substance is sensitive to moisture, high temperatures (thus it should be stored frozen) and low pH. In solutions with pH below 7 it decomposes in a few minutes. By testing we estimated acceptable stability of solution for lyophilization (pH about 12) to be not more than 24 hours if cooled and in nitrogen atmosphere. However together with excipients in form of lyophilized powder active substance is stabilized enough to meet more than two years of shelf life at temperatures up to 25°C.

• Formulation

All excipients used were of Ph. Eur. and USP grades. The active ingredient was formulated in 0,25 and 0,75 mg/mL in glycine buffer solution together with mannitol, sodium chloride and sodium hydroxide. The pH of solution was adjusted to approximately 12. The rubber stoppers tested were selected from Helvoet Pharma (FM257 and FM460) and West (B2-42). The aliquots of 2 mL were filled into 15 mL vials partly closed with autoclaved rubber stoppers (121°C, 30 minutes) and lyophilized by prescribed lyophilization process. After lyophilization the vials were closed in nitrogen atmosphere and equipped with aluminum flip-off cap to provide permanent sealing.

• Testing methods

Loss on drying (LOD) –To determine residual moisture, stoppers were first autoclaved at 121°C for 30 minutes (autoclave Kambič) and optionally dried for 6 hours at 110°C (hot air sterilizer, Kambič). Residual moisture of rubber stoppers was evaluated by measuring of loss on drying at 105°C in vacuum for 4 hours in the presence of P_2O_5. For lyophilized powder, loss on drying was determined at 70°C for 4 hours in the presence of P_2O_5.

pH – The pH of lyophilized product was evaluated by measuring the pH of reconstituted lyophilized cake in 2 mL of water for injection.

Stability testing – Stability of the product was evaluated at 60°C and 80°C after storage for 7 days. To determine the rubber CO_2 permeability effect on the product pH and degradation (Fig. 3) CO_2 atmosphere was additionally applied by allowing dry ice to evaporate inside a sealed box along the product. The amount of degradation was measured by an UPLC method with detection at 200 and 210 nm.

Modeling – samples with different stoppers were exposed to various stress conditions and the amount of the main degradant measured as an indication of product stability. In addition, pH and LOD were measured for each sample at each condition. Using measurements from 80°C (under air and under CO_2 atmosphere), a bivariate linear model was built to fit the degradant amount based on pH and LOD as the predictor variables. The fit was very good ($Radj^2$=0.99 at 7 degrees of freedom) and allowed an accurate prediction of degradation based on pH and LOD. Based on the simulation from this model, appropriate limits were set for LOD and pH (such that the amount of degradation at 80°C for 1 week would not exceed the reference value of 15%).

RESULTS

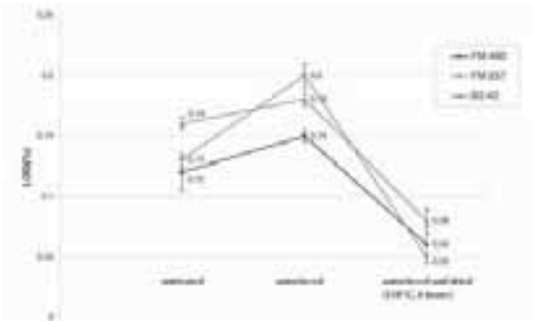

Figure 1: Amount of rubber stopper residual moisture: untreated, after autoclaving and after drying.

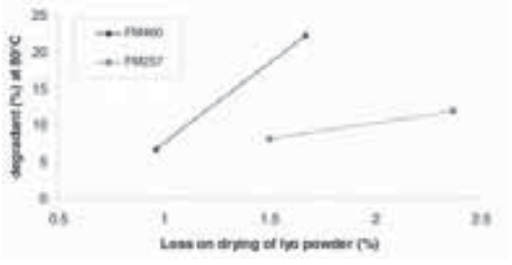

Figure 2: Amount of degradation product with respect to rubber stopper residual moisture.

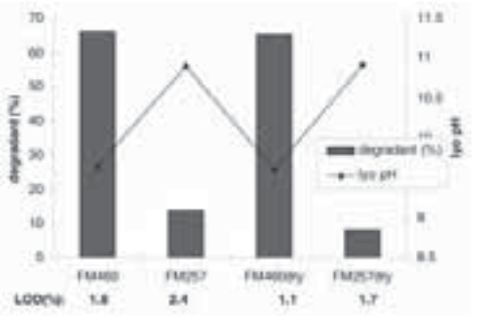

Figure 3: Effect of product pH and moistur (LOD) on drug product degradation (80 °C, CO_2, 7 days).

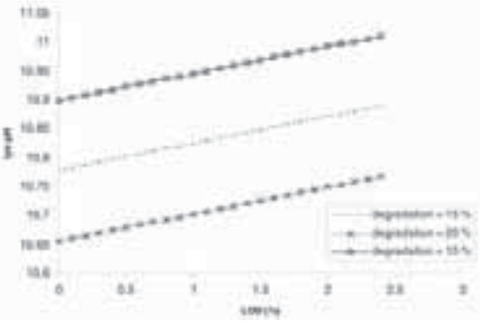

Figure 4: Simulation of 10%, 15% and 20% degradation with respect to pH and product moisture (LOD).

CONCLUSION

We confirmed the correlation between rubber stoppers residual moisture and product degradation. For ultimate stability of this product the rubber stoppers should be additionally dried after autoclaving. Because of pH dependent stability of active ingredient, maintaining high pH during shelf life is crucial. For such lyophilized products the effect of rubber stoppers CO_2 permeability has to be considered. We concluded that the influence of pH value on the degradation is about 20 fold higher than the moisture content. To reach desired stability we have to retain high pH and low moisture content in the lyophilized product. Appropriate specification limits for pH and loss on drying were set based on the predictions of drug degradation, gained from the bivariate model.

REFERENCES

1. Templeton AC, Placek J, Xu H, et al. Determination of the Moisture Content of Bromobutyl Rubber Stoppers as a Function of Processing: Implication for the Stability of Lyophilized Products. PDA J of Pharm Sci and Technol. 57(2): 75 – 87 (2003).
2. Corveleyn S, De Smedt S, Remon JP. Moisture absorption and desorption of different rubber lyophilization closures. Int J Pharm. 159: 57 – 65 (1997).

PO046

Assessment of blend homogeneity with tablet content uniformity testing as an alternative evaluation of granulation process capability

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INTRODUCTION

Batch homogeneity of the blend represents a challenging part in quality assessment in the production of solid dosage forms. The method to demonstrate that the active ingredient is uniformly distributed across the whole batch has to be reliable and simple for routine production. The efficiency of the granulation process is still mostly demonstrated by blend uniformity analysis (BUA). It is well known that sampling of a blend plays a critical part in blend uniformity assessment.

As it is recommended by FDA the blend uniformity analysis should be performed routinely as in-process test for commercial batches.

To demonstrate the efficiency of the granulation process and blend uniformity, samples are taken with specific sampling thief that enables taking samples in predefined quantity and locations. It is well known in practice that such sampling technology is not always capable to take a representative sample. Differences in particle size distribution, shape, density and electrostatic charge of the blend´s ingredients, i.e. excipients and active substances, can have impact on blend sampling (1). Sampling error is usually introduced with sampling the blends of lower drug content or when specific electrostatic properties of a sampling powder exist (1).

The alternative method is described in FDA draft guidance: The Use of Stratified Sampling of Blend and Dosage Units to Demonstrate Adequacy of Mix for Powder Blend" (2). This approach enables to assess the blend homogeneity with content uniformity results that are weight corrected. The acceptance criteria for weight corrected results are that mean value is within 90.0 – 110.0% with RSD not more than (NMT) 4.0% (2).

A case study presented is showing that demonstrating the homogeneity of the granulate can be efficiently done by the content uniformity of tablets by stratified sampling instead of unreliable sampling technique of granulate with a sampling thief.

The aim of this study is to present with statistical methods that the variability of the blend uniformity can be estimated efficiently by the content uniformity analysis where the weight variation is eliminated. The statistical evaluation, specifically moving range and six sigma approaches are used to demonstrate that the content uniformity testing on tablets including the weight correction is a well established method and can be used as in process control for demonstration of blend uniformity.

MATERIALS AND METHODS

• Materials

13 batches of a drug product A were manufactured. Granulate blends with the same composition were compressed into 4 different strengths (10, 20, 40 and 80mg). 10 and 20 mg batches were manufactured on pilot scale, 40 and 80mg batches on commercial one. All blends contain 8.3% of a drug content.

• Methods

Sampling of a blend was performed by sampling thief which enables the sampling in the size of 1- 3 times of dosage unit weight. Samples were taken in a shape of a slightly compressed form at 10 predetermined locations in the blender.

For content uniformity tests tablets were taken from 20 stratified locations, uniformly spaced during compression process. 3 tablets per each location were sampled (60 tablets per batch). Totally 780 analysis have been performed by HPLC method.

Assessment of granulation process capability has been performed with content uniformity results that were weight corrected to eliminate the effect of potentially variable tablet weight on measurement of blend adequacy (2). Each content uniformity result was weight corrected with the following equation:

$$ASSAY^{*}_{CORRECTED} = \frac{(ASSAY_{ACTUAL} \times WEIGHT_{TARGET})}{(ASSAY_{TARGET} \times WEIGHT_{ACTUAL})} \times 100\ \%$$

* expressed as % of label claim

Population standard deviation σ, population mean μ, UCL and LCL with 95% confidence interval were calculated (3). Estimation of granulation process variability was performed with moving range control charts where variability between sampling points is considered and sampling standard deviations for XmR charts are calculated from between-subgroup variability, in our case between 20 locations during compression process (4). Capability analyses were done with statistical six sigma approach to demonstrate high efficiency of alternative blend uniformity assessment (5).

RESULTS AND DISCUSSION

The blend uniformity results of 13 batches, obtained by sampling thief, show high variability. 130 individual results of BUA vary from 83.8% to 99.3%. Although all batches meet the criteria that individual values are within ±10% of the mean and that relative standard deviations (RSD) are below 5.0%, high variability is noticed within some batches (RSD up to 3.569%) and between batches (RSD from 0.444% to 3.569%). The variability of blend uniformity analysis could demonstrate an insufficient granulation process or significant impact of the sampling error.

Significant differences between the blend uniformity and tablets content uniformity results that were weight corrected are observed at pilot and commercial scale. RSD values of content uniformity are much lower compared to the RSD of the blends, varying from 0.471 to 1.375. Additionally the average results of the blend uniformity are significantly lower than the content uniformity of tablets. An example of 40 mg strength is presented (Figure 1):

A. Blend: sample average$_{10}$=93.30%, RSD$_{10}$=2.575%, estimated σ=3.952%, estimated population upper mean=95.34% and estimated lower population mean=91.26%;
B. Tablets content uniformity weight corrected: sample average$_{60}$=98.98%, RSD$_{60}$=0.571%, estimated σ=0.668%, estimated upper population mean=99.18% and estimated lower population mean=98.78%.

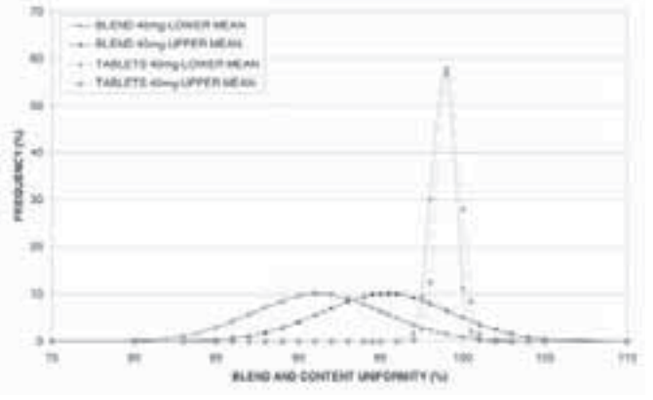

Figure 1: Blend uniformity and content uniformity normal distribution for upper and lower population mean for 40mg strength.

The difference between the blend uniformity and tablets weight corrected content uniformity results is significant. Higher values of content with less variability are observed for weight corrected content uniformity results. Based on blend uniformity results wrong conclusion considering the batch uniformity could be taken. Considering the criteria 90.0-110.0%, only 62.52% of the whole population of 40mg blend batch would pass. In our case the blend uniformity assessment using the sampling thief can therefore not represent an appropriate sampling method. Evaluation of variability between 20 uniformly spaced locations during compression has been performed with statistical method of moving range (4). Weight corrected content uniformity averages are within the calculated values of moving range control chart. The evaluation of variability between sampling points demonstrate that the drug is uniformly distributed. It could be concluded that estimation of efficiency of blending process can be reliably performed with moving range approach and weight corrected content uniformity results.

The capability of granulation process has been presented also with six sigma statistical tool (5). We have demonstrated an excellent capability of granulation process at the level of six sigma considering the specification limits 90.0% -110.0%. Using weight corrected results of content uniformity we have indirectly demonstrated the homogeneity of a blend.

CONCLUSION

Sampling of the blend with a thief can not always represent an appropriate method for blend uniformity assessment and may therefore lead to the wrong conclusions regarding the process capability (6).
The alternative method of stratified sampling of the tablets providing weight corrected content uniformity results can be recognized as an appropriate and valid method for demonstrating the homogeneity of the blend and efficiency of granulation process.

References

1. J. Berman, The Compliance and Science of Blend Uniformity Analysis, PDA J.Pharm. Sci. Technol. 55: 209-222 (2001).
2. FDA Draft Guidance for Industry, Powder Blends and Finished Dosage Units – Stratified In-Process Dosage Unit Sampling and Assessment, CDER, FDA, (2003).
3. P. Cholayudth, Simple Conversion Between Lot vs. Sample Statistics in Pharmaceutical Dosage Uniformity, J.Valid.Technol. 11: 116-126 (2005).
4. F.W. Breyfogle, XmR Charts: Individual Measurements. Implementing Six Sigma: Smarter Solutions Using Statistical methods, John Wiley & Sons, Inc., New Jersey, 226-230 (2003).
5. D.C.Montgomery, Modified and Acceptance Control Charts. Introduction to Statistical Quality Control, John Wiley & Sons, Inc., New Jersey, 429-434 (2005).
6. B.Rihtaršič, Evaluation of Process Capability of Granulation With Content Uniformity Testing, MSci thesis, University of Ljubljana, Faculty of Pharmacy (2007).

PO047

Tools of process analytical technology for predicting quality of drug layered and coated pellets

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INTRODUCTION

Coated pellets as multiparticulate dosage form offer several advantages related to safety and effectiveness of the medicinal product such as reproducibility of gastric emptying and of absorption, and predictable plasma levels with lower probability of dose dumping due to modified release [1-3]. This basic concept of multiple-unit systems is the fact that the dose of the active ingredient is released by the individual subunits, and the functionality of the entire dose depends on the quality of the subunits [4].

Layered pelletizing involves a process in which drug in powder, solution, or dispersion form is loaded onto inert starting cores [5]. Starting pellets (neutral or inert pellets, beads, seeds, spheres, nonpareils) have several benefits, such as they serve as nuclei with standardized shape, therefore the product shows more exactly defined surface for functional coating [6]. Pelletization with them is much more easier, because the critical manufacturing step, the wet agglomeration of nuclei can be neglected, and the preformed nuclei are spherical. Today, neutral pellets with narrow size distribution are available as materials manufactured from sugar, microcrystalline cellulose, and isomalt [7].

The objective of this study was to investigate the effects of formulation and manufacturing parameters on the layering and coating process using 3 types of neutral pellet cores. The processes were followed by near-infrared (NIR) spectroscopy and image analysis and the prepared samples were characterized taking the physical characteristics and drug dissolution profile into consideration.

EXPERIMENTAL METHODS

Preparation of the pellets

Sodium diclofenac and sodium ibuprofen (Sigma-Aldrich Chemie GmbH, Germany) served as model drugs. Core materials: sugar (Pharm-a-spheres®, H.G.Werner GmbH, Germany), microcrystalline cellulose (Ethispsheres®, NPPharm Ltd., France) and isomalt (galenIQ™, BENEO-Palatinit GmbH, Germany). Drugs were loaded on neutral pellets using 2% hydroxypropyl-methylcellulose (Pharmacoat 606, Shin-Etsu Chemical Co., Japan) binder solution. Drug layering and coating were performed by bottom spraying in fluid bed equipment (Aeromatic Strea-1 Aeromatic-Fielder AG, Switzerland; and MiniGlatt 4, Glatt GmbH, Germany). Coating systems contained Eudragit® (Degussa, Germany) RL /RS types, triethylcitrate ((Fluka Chemie AG,

Switzerland), and micronized talc (Sigma-Aldrich Chemie GmbH, Germany).

NIR diffuse reflectance spectroscopy
The diffuse reflectance of pellets was measured by Hitachi U-3501 UV/VIS/NIR spectrophotometer equipped with integrating sphere (d = 60 mm) and PbS detector. The reflectance was recorded in the 200-2500 nm wavelength range using a 5 mm layered cell.

Image analysis
Image analysis was conducted on samples of 200 pellets using a system consisting of a stereomicroscope (SMZ 1000 type, Nikon, Japan), a fiberoptic light source (Intralux 5000-1 type, Volpi, Switzerland) a digital camera (Coolpix 4500 type, Nikon, Japan) and the software Image Pro Plus 4.5 (Media Cynerbetics, USA).

Physical characteristics
Physical characteristics (density, hardness, friability) of pellets were tested according to adopted Eur. Ph. methods.

Wettability studies
The contact angle and liquid adsorption of samples was determined using KSV Sigma 70 (KSV Instrument Ltd., Finland) instrument with powder wettability measuring device in the applied binder solutions and coating dispersions.

Drug dissolution test
For the determination of dissolution profiles of pellets the rotating basket method of Eur. Ph. was applied at 100 rpm, at 37 ± 0.5ºC temperature (Hanson SR8-Plus™, Hanson Research, USA). Studies were conducted in 900 ml of dissolution medium at pH=1.2, pH=4.5 and pH=6.8.

RESULTS AND DISCUSSION
The drug layering and functional coating processes were monitored by image analysis of pellets considering their size and shape (Figure 1). The shape of pellet samples was characterized as roundness=perimeter2/(4 Area), where the values were close to 1 for each type of neutral pellets. A slight decrease was observed in the roundness during the layering, but this bias was balanced by coating.

All of pellets demonstrated satisfactory flowability, hardness and friability as well as wettability in the used binder liquids.

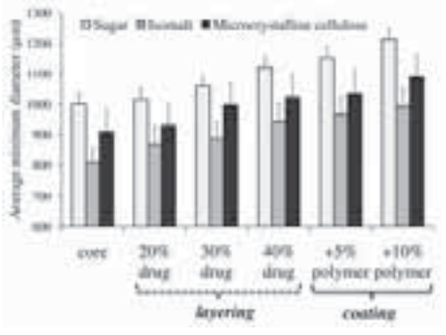

Figure 1: Changes in size of pellets during drug layering with sodium ibuprofen and coating with Eudragit RL/RS (±SD, n=200 pellets)

Both layering and coating of pellets can be followed by recording NIR diffuse reflectance spectra. Figure 2 demonstrates the changes in the spectra during the coating process of of 20%, 30%, and 40% sodium ibuprofen onto neutral pellets (galenIQ™ 980) at 5% and 10% level of Eudragit RL/RS polymer. Dissolution profiles of sugar and isomalt based pellets were similar demonstrating the same release mechanism in the case of water soluble pellet cores.

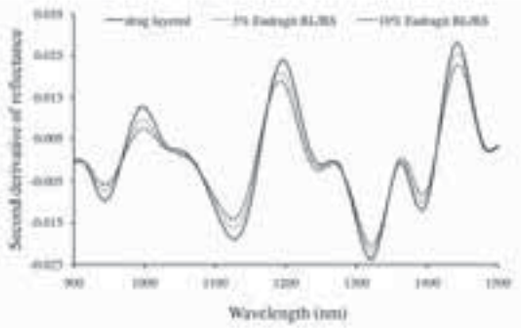

Figure 2: Changes of NIR spectra of pellets during the coating process

CONCLUSION
To understand and control manufacturing process of pellets, NIR spectroscopy and image analysis may serve as nondestructive test methods to characterize the changes during drug layering and functional coating. Beside type and amount of coating polymers, the dissolution mechanism from layered pellets can be influenced by the type of neutral spheres, too.

REFERENCES
1. Schultz P., Kleinebudde P. A new multiparticulate delayed release system. Part I : Dissolution properties and release mechanism. J. Control. Rel. 47: 181-189 (1997).
2. Dévay A., Mayer K., Pál Sz., Antal I: Investigation on drug dissolution and particle characteristics of pellets related to manufacturing process variables of high-shear granulation. J. Biochem. Biophys. Meth. 69: 197-205 (2006).
3. Musko Z., Bajdik J., Pintye-Hodi K., Szabo-Revesz P., Kelemen A., Eros I. Preparation of pellets containing theophylline. Pharm. Ind. 64: 1194-1198 (2002).
4. Chopra R., Alderborn G., Podczeck F., Newton J.M., The influence of pellet shape and surface properties on the drug release from uncoated and coated pellets, Int. J. Pharm. 239: 171–178 (2002).
5. Sinchaipanid N., Chitropas P., Mitrevej A. Influences of Layering Process on Theophylline Pellet Characteristics. Pharm. Dev. Tech. 9: 163-170 (2004).
6. Werner D. Sugar spheres: a versatile excipient for oral pellet medications with modified release kinetics. Pharm. Tech. Eur. 18: 35-41 (2006).
7. Heinicke G., Schwartz J.B.: Particle size distributions of inert spheres and pelletized pharmaceutical products by image analysis. Pharm. Dev. Tech. 9: 359-367 (2004).

PO048

Preparation, characterization, and in vitro antibacterial activity of Mangosteen crude extract with the storage stability of antibacterial film

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INTRODUCTION

Mangosteen, in the scientific language as *Garcinia mangostana Linn.*, is known as the "Queen of fruits" in Thailand. Compounds isolated from the fruit peel of mangosteen are contain abundant xanthones; especially, α-mangostin and it had been worldwide used as traditional medicine for anti-inflammatory, antibacterial and anticancer effects. Nowadays; it's popularly applied to cosmetic and pharmaceutical products. Because α-mangostin represent the majority of the clinical benefits of this herbal medicine, therefore it is reasonable and logical to determine the concentration of α-mangostin as chemical marker for the quality control of *G. mangostana* and its products. Recently, in our research concerning with evaluation on products of mangosteen such as antibacterial film prepared from mangosteen peel extract. However, there is little information for quality and quantity determination of α-mangostin in mangosteen and stability in antibacterial film. This method was fully validated according to International Conference on Harmonization (ICH) which would serve as stability- indicating assay method for α-mangostin in presence of their degradation products. Furthermore, the sensitivity was evaluated for its application which was expected that this method would be efficient in analyzing low concentration of α-mangostin in antibacterial film prepared from mangosteen peel extract. Evaluation of antibacterial activity was also demonstrated.

EXPERIMENTAL METHOD

• Preparation of mangosteen peel extracts

Dried powder of *G. mangostana* was extracted by refluxing with each organic solvents; acetone, dichloromethane, ethanol (95%), ethyl acetate, and acetone:dichloromethane (1:1, v/v) for 1 h. The extracts were filtered and evaporated to dryness by rotary evaporation under reduced pressure to yield the crude extracts. These extracts were dissolved and transferred to 25-ml volumetric flask and then diluted to final volume with methanol. Two milliliters of solutions were pipetted and transferred to 10-ml volumetric flask and then diluted to final volume with methanol. All solutions were filtered through 0.45 mm membrane filter before injected to HPLC system.

• Stability indicating assay

Forced degradation of α-mangostin was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress condition. Thermal (in a controlled-temperature oven at 80°C for 3 h) and photo-degradation (under UV radiation having peak intensities at 254 and 366 nm for 6 h) were preceded in solid state. After degradation stock solutions were prepared by dissolving in methanol to achieve final concentration of 25 mg/ml. For hydrolytic and oxidative degradation, solutions were prepared by dissolving a-mangostin in extract in small volume of methanol and later three dropped with 3% hydrogen peroxide (3% H_2O_2), 3N HCl, or 3N NaOH solution and heated at 80°C for 3 h. After degradation this stock solution were prepared by dissolving in methanol to achieve final concentration of 25 mg/ml. All the samples solution for acid/base hydrolysis and oxidative were kept in a dark to prevent the effect of light.

• HPLC method validation

This method was fully validated according to ICH of note for guidance on validation of analytical procedures on specificity, linearity, recovery, accuracy, precision, limit of detection and limit of quantification [1]. The HPLC system consisting of a quaternary solvent delivery system, autosampler, solvent degasser and ultraviolet detector. The quantification wavelength was set at 240 nm. Chromatographic separation was carried out at room temperature using C_{18} analytical column with C_{18} guard column. The isocratic mobile phase consisted of 0.2% formic acid–acetonitrile (30:70, v/v) which was pumped at a flow rate of 1.0 mL/min. The injection volume was 20 μL.

• Evaluation of antibacterial activity against *Staphylococcus* spp. and *Propionibacterium acnes*.

The mangosteen peel extract was subjected to antibacterial activity evaluation. Disc diffusion method was used to test the susceptibility for antibacterial activity against *S. aureus*, *S. epidermidis and P. acnes.* This experiment was performed by the method of National Committee for Clinical Laboratory Standard [2] with some modifications. *S. aureus* and *S. epidermidis* were streaked on Mueller-Hinton Agar (MHA) while *P. acnes* was streaked on Brain-Hard Infusion (BHI) supplemented with 1.5% agar-agar**.** A sterile paper disc was impregnated with the mangosteen peel extract (concentration 2.0, 1.0 and 0.5 mg/disc) and then disc was applied and pressed down on the agar. Control disc were similarly prepared using DMSO as a negative control and standard tetracycline disc as a positive control. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. The minimum inhibition concentration (MIC) value was determined using macro agar dilution assay [3]. The results were expressed as the lowest concentration of standard and the extract that produced a complete suppression of colony growth. The minimum concentration (MIC) was defined as the lowest concentration of the compound to inhibit the growth of microorganisms.

• Stability of antibacterial film

The stability of α-mangostin, the main constituent of crude extract, were evaluated during the storage of antibacterial films made by our labora-

tory by HPLC analysis of film extracts following storage from 0 to 120 days at 4, 30 and 45 degrees Celsius (humidity-control).

RESULTS AND DISCUSSION

The comparison of efficiencies of the different extraction solvents is based on the determination of α-mangostin from *Garcinia mangostana* Linn. by RP-HPLC. From the results indicated that the dried powders of mangosteen extracted by refluxing with dichloromethane showed the high-yielding extracts for α-mangostin (47.46% w/w). Therefore, dichloromethane was selected as solvent for extraction the dried powders of mangosteen. HPLC studies under different stress conditions indicated the following degradation behaviors. It was found that α-mangostin was stable to light, heat and basic hydrolytic under conditions used. Nevertheless, the α-mangostin demonstrated the decomposition in acidic hydrolytic and oxidative condition but the degradation products have no interference with this analytical method. Therefore, this proposed method is also stability-indicating assay. The assay was fully validated and shown to be linear ($r^2 > 0.999$) for the analytical range of 1.0-20.0 μg/ml, sensitive (LOD = 0.02 μg/mL and LOQ = 0.08 μg/mL), accurate (intra-day was between 98.1-100.8%, inter-day was between 90.0-101.3%), precise (intra-day variation ≤ 1.8%, inter-day variation ≤ 4.3%), specific and good recovery (recovery rates ≥ 95.8%). Total analysis was about 8 min with typical retention time of α-mangostin of about 6 min. From the results of disc diffusion method indicated that the dichloromethane extract at the concentrations of 0.5, 1.0, and 2.0 mg/disc could effectively inhibit the growth of all bacteria tested. Determination of MIC value demonstrated that this extract showed the strong antibacterial activities against *S. aureus, S. epidermidis*, and *P. acnes* with MIC values of 3.90, 2.00, and 3.90 μg/ml, respectively. None of the antibacterial films showed a significant loss of α-mangostin during the 120 days storage period at either temperature.

CONCLUSION

The method showed simplicity, good linearity, high precision & accuracy and good recovery of compounds of interest. This analytical method was proved to be a validated stability-indicating assay for assay of α-mangostin in the presence of degradation products. The present method should be useful for analytical research and for routine quality control analysis of α-mangostin in mangosteen peel extract and products of mangosteen such as antibacterial film prepared from mangosteen peel extract. Furthermore, the results suggested that *G. mangostana* extract possessed antibacterial activities against *S. aureus, S. epidermidis*, and *P. acnes* through the active constituent, α-mangostin. Therefore, mangosteen peel extract would be an interesting material for further study on an alternative treatment of acne.

REFERENCES

1. International conference on harmonization of note for guidance on validation of analytical procedures: text and methodology, Step 5 of the ICH Process, 1995.
2. National Committee For Clinical Laboratory Standards. Performance standards for antimicrobial sus-ceptibility tests for bacterial that grow aerobically. Approved standard M7-T2. 2nd ed NCCLS, 1993.
3. Lorian, V: Antibiotics in Laboratory Medicine 4th Ed. Williams and Wilkins, 1996.

PO049

Dissolution of bioactive pharmacons from sustained release dosage forms

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INTRODUCTION

The release of pharmacon from a dosage form is a substantial parameter of a pharmaceutical preparation. Commonly used analytical detection methods based on UV absorption can characterize the dissolved amount of an active ingredient from a delivery system however they are not able to give any information about biological activity of the drug released in case of bioactive pharmacons. The Microbiologically Detected Dissolution (MDD) test can follow the drug release by detecting changes in the antimicrobial activity of a bioactive pharmacon as a function of time.

They were chosen from four different microbiologically active antibiotic groups (fluoroquinolones, macrolides, tetracyclines and fungicides) (Table 1).

There is a commendation of NIH (National Institute of Health) to give biological activity of samples at different concentration.

There is a poor correlation between concentration measured by spectrophotometric method and biological activity measurement for example at polyen antibiotics, nystatine (Horváth és Koczka, Nature, 1964).

EXPERIMENTAL METHODS

Test strains: Bacillus subtilis, ATCC 6633, Escherichia coli, ATCC 25922 and Candida albicans, ATCC 90028.

Biological activity of samples was determined on Silica gel TLC plates by MDD (Microbiological Dissolution Detection) method. Pharmacon concentration was determined spectrophotometrically by an UV-VIS spectrophotometer (Jasco V 550, Jasco Co., Tokyo, Japan) Dissolution test of manufactured tablets was carried out in triplicates in Erweka DT 700 apparatus (Heusenstamm, Germany) using 900 cm^3 deionised and degassed water and 0.1M HCl at 37°C applying the paddle method of Ph.Eur at 100 min^{-1}.

Table 1: Major characteristics of the studied antimicrobial compounds

Type of antimicrobial drugs	Examined antimicrobial groups	Examined antimicrobial compounds	Microbial effect	Effective on type of microbe
Chemotherapeutics	Fluoroquinolones	Ciprofloxacin	Microbicide	Gram-negative
Antibiotics	Macrolides	Clarithromycin	Bactericide/bacteriostatic	Gram-positive, intracellular microbes
Antibiotics	Tetracyclines	Doxycycline	Bacteriostatic	Gram-negative, Gram-positive, intracellular microbes
Antibiotics	Fungicides	Nystatin	Fungicidic/fungiostatic	Fungi

Determination of diameter of inhibition spots was determined by analyzing of brightness distribution on TLC plate: 300 dpi scanner. Zeiss Axio Imager A1, Carl Zeiss, Jena, Germany. Software AxioVision Rel. 4.5.

RESULTS AND DISCUSSION

Microbiological detection for two different types of bacteria (B. subtilis and E. coli) are shown on Figure 1-2 and Table 2.

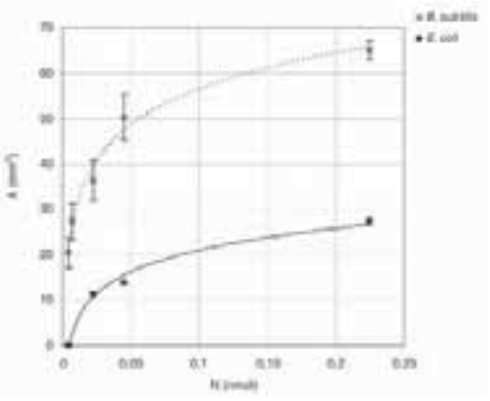

Figure 1: Function graphs between inhibition spot area (A, mm^2) and amount of doxycycline (N, nmol).

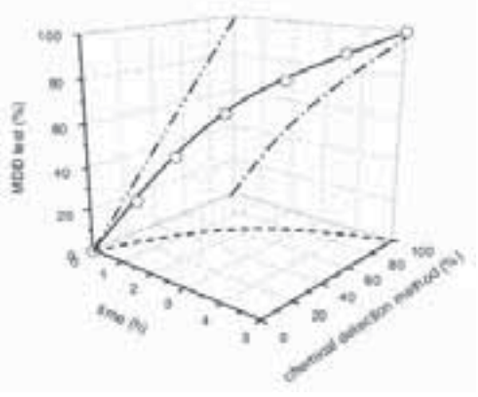

Figure 2: 3D drug delivery system (1.) of doxycycline detected by chemical (2.) and MDD (method and correlation between two methods (4).

Table 2: Range of studied antibiotics and correlation between logarithm of amount of applied antibiotics and area of inhibition spot using B. subtilis as test bacterium (^{x}test organism : Candida albicans).

Antibiotics	Amount of antibiotics (nmol) on TLC plate	Coefficient of correlation (r^2) between logarithmic amount of applied antibiotics (nmol) and area of inhibition spot (mm^2)
ciprofloxacin	0.003 – 0.12	0.875 (linear); 0.928 (exponential)
clarithromycin	0.040 – 1.34	0.966 (linear)
doxycycline	0.045 – 0.45	0.992 (linear)
oxytetracycline	0.001 – 1.00	0.944 (linear)
nystatin^x	0,01 – 1,00	0,993 (linear)

CONCLUSIONS

Applying UV spectrophotometry or UV detection after HPLC separation only the amount of dissolved bioactive pharmacon is detected by traditional methods. Classic biological methods are too consumptive of time and labor-intensive. The MDD technique gives further new complementary information on the dissolution kinetics of bioactive pharmacon.

MDD method could give new predictive test possibilities for antimicrobial activity in project of new drug delivery systems. The stability of dissolved bioactive pharmacon can be followed by MDD directly on chromatoplate after TLC separation.

References

1. Horvath, I., Koczka, I. Nature 203: 1305-1306 (1964)
2. Bershtein I. Ia., Spectrophotometric determination of nystatin based on Ashtron and Tootill's method, Antibiotiki 25: 444-9 (1980)
3. Dévay, A., Uderszky A.J., Rácz, I., Investigation of the liberation of the active ingredient from pharmaceutical microcapsules on the basis of RRSBW distribution. Acta Pharmceutica Hungarica 53: 28-35 (1983)
4. Botz, L., Kocsis, B., Nagy, S., Bioautography (in Bioassays), in Paul J. Worsfold, Alan Townshend and Colin F. Poole (Eds.) Encyclopedia of Analytical Science, Second Edition Elsevier, Oxford, Vol,1, pp. 271-277 (2005)
5. Dévay, A., Kocsis, B., Pál, Sz., Bodor, A., Mayer K., Nagy S., New method for microbiological detection of delivery process from dosage forms containing antibiotics, European Journal of Pharmaceutical.Sciences, 25/Suppl.1:S81-S83 (2005).

PO050

Extraction and Characterization of *Spartium junceum L.* seed oil

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INTRODUCTION

Spartium junceum L. is a small shrub in the Mediterranean countries; in Italy it grows spontaneously and it is very widespread. It is even more abundant in the central and southern Italy, where it is a typical part of the landscape. This plant usually grows at the sides of country roads in areas exposed to sunlight.

The Spartium junceum L. seeds contain an oil that has not been characterized and uses have not been suggested. This communication reports the extraction and physico-chemical properties of the Spartium junceum seed oil.

EXPERIMENTAL METHODS

Extraction of the oil

The oil was extracted by cold pressure and was analysed by standard methods for oil and fat analysis. Refractive index was measured with an Abble refractometer at 20°C.

RESULTS AND DISCUSSION

The Spartium junceum L. seed oil was yellow in colour and remained liquid at room temperature.

The physico-chemical properties of Spartium junceum oil are shown in Table 1.

The oil showed high values of acidity, iodine and saponification. The high acid value and free acidity showed that the oil requires refining before use for food industry and edible purposes.

Table 1: Physico-chemical properties of Spartium jiunceum L. oil

Parameter	Spanish Broom oil
Acid value (mg/g)	19.19
Free fatty acid (as oleic %)	9.65
Peroxide value	15.00
$K_{230\ nm}$	4.18
$K_{270\ nm}$	2.11
Iodine value (g/100 g)	134.20
Saponification value (mg/g)	194.83
Refractive index (20°C)	1.4750

The oxidative state of Spartium junceum L. seed oil was determined using the peroxide value and specific extinction at 232 and 270 nm. $K_{232\ nm}$ is related to the degree of primary oxidation of the oil, whereas $K_{270\ nm}$ is related to the secondary oxidation products. The K_{232} value was higher than $K_{270.}$ The saponification value of the oil suggested that it could be used in soap and shampoo production, while the iodine value suggested a use in the resin formulation.

PO051

Selection of the appropriate filter for the sterile filtration of a solution containing a macromolecular active ingredient

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INTRODUCTION

Macromolecules are present in several drug formulations as a part of a drug delivery system where they regulate the dissolution of the active substance from the dosage form. In some special cases they can also be the active ingredients of drug products, for instance: treatment of osteoarthritis, wound healing, ophtalmology and treatment of cystitis. Some of the drug products used for the previously listed applications are sterile liquid preparations which are administered locally as injections, eyedrops or other way. Polymer molecules applied for this purpose are natural components of the tissues, and possess desirable biocompatible properties, furthermore they are bioadhesive. They are also involved in cell signaling and due to the chemical structure they show high viscosity and elasticity.

High temperature steam sterilisation is considered to be the safest and most practical means of sterilising medical devices and fluids. In the case of solutions containing a heat sensitive polymer, however, it cannot be applied, as autoclaving at high temperature can cause the degra-

dation of the polymer molecule and the decrease of its average molecular weight [1,2]. To prevent this phenomenon, sterilisation of the polymer solution can be performed by filtration at room temperature. Sterile filtration of a polymer solution through a bacteria-retentive 0.22 micron pore-sized filter can be complicated due to the large size of the macromolecule. Based on the literature, filter material and the structure of the pores have a considerable effect on the filterability of the molecules and particles larger than the nominal pore size [3].

The aim of the present study was to find the appropriate filter type at the early stage of the development of a solution containing a macromolecular active ingredient. Avoiding ultrafiltration and separation of the polymer was of high concern throughout the work.

MATERIALS AND METHODS

• Materials

The following filter membrane discs were tested:
Pore size: 0.45 micron+0.22 micron, Diameter: 47mm
Polyvinylidene fluoride (PVDF), Millipore (Budapest, Hungary)
Polyethersulfone (PES); Sartorius (Goettingen, Germany)
Cellulose acetate; Sartorius (Goettingen, Germany)
Pore size: asymmetric prefilter+0.22 micron filter,
Diameter: 47mm
Polyethersulfone(PES); PALL (Wien, Austria)
Polyethersulphone (PES) prefilter; polyethersulfone and polyvinylidene fluoride (PES+PVDF) 0.22 micron filter; PALL (Wien, Austria)

• Viscosity measurements

The intrinsic viscosity of the polymer was measured at 30°C applying a Lauda automatic viscometer having four Ubbelohde capillaries, a Processor Viscosity System 1 and a thermostat (D20 KP) (Lauda-Königshofen, Germany).

RESULTS AND DISCUSSION

During the experiments a 0.1 w/v% aqueous polymer solution was filtered through different filter membrane types with a diameter of 47 mm at room temperature. Each examined filter membrane consisted of a prefilter and a final filter (0.22 micron pore size) element. The reason for the application of the prefilter was to improve the spatial settlement and filterability of the macromolecules through the 0.22 micron pore-sized filter.

We found that the filtration of the polymer solution at room temperature could not be performed applying 2 bar pressure at the beginning of the process, because it resulted in the dramatic decrease of the filtration rate due to the clogging of the filter pores by the macromolecules. Therefore filtration was started at low pressure (0.5 bar), which was gradually increased up to 2 bar. The filtration rate decreased during the filtration procedure in the case of each tested filter.

The average filtration rate, and the amount of the solution, which was possible to be filtered through the different membranes are summarized in the table below (Table 1).

Filters having the highest filter capacity (italic) were further evaluated considering the change of the filtration rate as a function of the filtered solution quantity during the filtration procedure.

Table 1: Test results of the different filter membrane types

Filter type	Maximum amount of the filtered solution (ml)	Average filtration rate (ml/sec)
PVDF 0.45+0.22 micron	60	0.070
PES 0.45+0.22 micron	70	0.055
Cellulose acetate 0.45+0.22 micron	*100*	*0.035*
PES asymmetric prefilter + PES 0.22 micron	*100*	*0.051*
PES asymmetric prefilter + PES and PVDF 0.22 micron	60	0.031

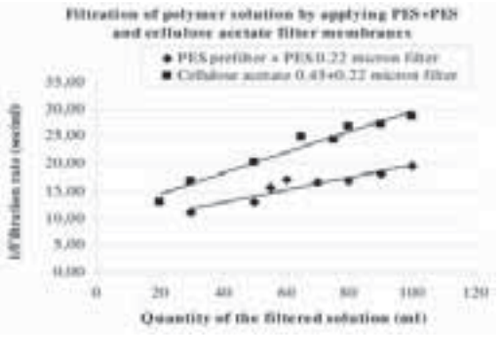

Figure 1: Reciprocal of filtration rate plotted against filtered solution quantity in the case filters showing the highest capacity

According to the diagram (Figure 1) it can be concluded that the extent of decrease of the filtration rate (increase of its reciprocal) is lower in the case of the polyethersulfone asymmetric prefilter + polyethersulfone 0.22 micron filter than for the other examined filter (cellulose acetate) having the same capacity. These results have significance from the viewpoint of solution filterability and filtration time.

The presence of ultrafiltration was checked by organoleptic examination of the filter membrane at the end of the filtration. No signs of ultrafiltration could be observed on the membranes.

Intrinsic viscosity of the polymer was measured before and after filtration to evaluate the possible separation of the macromolecules by their size (See results in Table 2).

Table 2: Intrinsic viscosity test results

Filter type	Intrinsic viscosity before filtration (dl/g)	Intrinsic viscosity after filtration (dl/g)
PES asymmetric prefilter + PES 0.22 micron filter	28.1	27.1

The filtration process was realizable due to the elasticity of the macromolecules. The asymmetric structure of the chosen polyethersulfone prefilter membrane influenced the filterability of the macromolecules

favourably and also contributed to avoiding ultrafiltration and early filter clogging.

CONCLUSION

Based on the experiments we found that the filtration of a polymer solution through a 0.22 micron filter could be performed at room temperature without occurence of ultrafiltration and without separation of the macromolecules. Filtration rate and filter capacity were influenced by the material of the filter membrane and the structure of the prefilter membrane layer. Polyethersulfone asymmetric prefilter with polyethersulfone 0.22 micron filter was selected to serve as a basis of the further scale-up experiments.

References

1. Jarry C., Chaput C., Chenite A., Renaud M.A., Buschmann M., Leroux J.C. Effect of stem sterilization on thermogelling chitosan-based gels. Journal of biomedical materials research 58:127-135 (2001).
2. Zahraoui C., Sharrock P. Influence of sterilization on injectable bone biomaterials. Bone 25:63S-65S (1999).
3. Goldbach P., Brochart H., Wehrlé P., Stamm A. Sterile filtration of liposomes: retention of encapsulated carboxyfluorescein. International Journal of Pharmaceutics 117:225-230 (1994)

PO052

In vitro test systems to detect and characterize effect of excipients on ABC-transporter activity

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According to official definitions excipients are inert ingredients used as a vehicle or medium of administration for the medicinal agents in approved drug products. However, some of them have been shown to modulate drug delivery by interacting with ABC-transporters and affecting drug efflux.

ABC-transporter proteins are expressed at high levels in the small intestine and at pharmacological barriers (e.g. blood-brain-barrier). Due to their physiological localization they may play a crucial role in effective drug delivery and absorption. SOLVO Biotechnology is developing high throughput assays to investigate *transporter protein-excipient* interactions. Our data demonstrate that excipients selectively interact with ABC transporters *in vitro*, thus these molecules might be involved in the modulation of ADME/Tox properties of drugs.

We have shown that some cyclodextrins inhibit BCRP/ABCG2 activity and the mechanism is at least partly extraction of membrane cholesterol (Pal et al., 2007 JPET). Our results also show that *Cremophor EL* and *Tween 80* inhibit both MDR1/Pgp and BCRP ATPase activity. In addition, we will be presenting data on transporter interactions of *Pluronic* and *Reverse Pluronic* block copolymers.

ACKNOWLEDGEMENT:

This work was supported by Hungarian Grants: GVOP-3.1.1.-2004-05-0506/3.0; GVOP-3.1.1.-2004-05-0440/3.0; NKFP-1A/041/04; Asbóth-XTTPSRT1; GVOP-3.3.3-05/1.-2005-05-0118/3.0; GVOP-3.3.2.-2004-04-0001/3.0; Kozma - TUDAS_06-HTShu_

References:

Pál Á., Méhn D., Molnár É., Gedey S., Mészáros P., Nagy T., Glavinas H., Janáky T., von Richter O., Báthori G., Szente L., Krajcsi P., Cholesterol loaded insect cell membranes - improved *in vitro* model to study function of human ABCG2 **JPET 321:1085 2007**

PO053

The efflux pump inhibitory properties of (thiolated) polyallylamines

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INTRODUCTION

The oral bioavailability of many drugs is limited by various barriers: passage through the mucus layer, permeation across the intestinal membrane, first pass metabolism and efflux transporters such as P-glycoprotein (P-gp), situated on the apical membrane of the intestinal epithelium . It exhibits a very broad substrate specifity, transporting simple ions, complex lipids and xenobiotics. Thus, inhibition of efflux pumps will improve the uptake of drugs absorbed via this route. Besides low

molecular mass inhibitors polymers have been reported to act as inhibitors, offering the advantage of not being absorbed, thus avoiding systemic adverse effects. Especially thiolated polymers (thiomers) have been found to exhibit interesting characteristics, such as improved mucoadhesion, controlled drug release, permeation enhancing and enzyme inhibitory effects . It was the objective of this study to investigate if a new thiomer, namely a poly(allyl amine)-*N*-acetylcysteine conjugate (PAH-NAC), shows permeation enhancing and in particular efflux pump inhibitory properties. PAH is a cationic, non biodegradable polymer which is likely not at all absorbed from the GI-tract due to its high molecular mass. A cross-linked derivative is already on the market for the treatment of hyperphosphatemia in patients suffering from end-stage renal disease . PAH-NAC conjugates were characterized by determining the thiol group and disulfide bond content, and the efflux pump inhibitory effect using rhodamine-123 (Rho-123) as the model substrate for P-gp.

EXPERIMENTAL METHODS

• Synthesis

PAH (70 kDa) was cross-linked with dimethylsuccinate in dry methanol containing 15 % triethylamine at 80°C. After the mixture had cooled down to room temperature under stirring, the solid polymer was filtered and rinsed with isopropanol. The obtained polymer was then dispersed in demineralised water lyophilized (-78°C, 0.01 mbar, Benchtop 2K, VirTis, NY, USA).

PAH-NAC conjugates of three different molecular masses (cross-linked, 70 and 15 kDa) were synthesized via the formation of amide bonds mediated by a carbodiimide.

• Determination of thiol groups and disulfide bonds

The degree of the coupling reaction was evaluated by an iodometric titration method . The content of disulfide bonds was determined the same way, after reducing the bonds to free thiol groups with sodium borohydrate.

• Efflux pump inhibition studies

Efflux pump inhibition studies were performed with ileum tissue of freshly excised rat intestine in Ussing type diffusion chambers in HEPES buffer at pH 7.2. The permeated amount of Rho-123 was determined in the presence of 0.5% (m/v) of either polymer (PAH) or thiomer (PAH-NAC) and buffer only as control by fluorescence spectroscopy ($\lambda_{exc.}$= 485 nm, $\lambda_{emm.}$= 520 nm). Apparent permeability coefficients were calculated according to the following equation:

$$P_{app} = Q/(A*c*t)$$

P_{app} .... apparent permeability coefficient [cm/s]
Q .... amount permeated within incubation time [µg]
A .... diffusion area [cm^2]
c .... initial concentration in donor chamber [$\mu g/cm^3$]
t .... total incubation time [s]

Permeation improvement ratios were calculated according to the following equation:

$$R = P_{app}\ (polymer)/\ P_{app}\ (medium\ only)$$

RESULTS AND DISCUSSION

Table 1 shows the amount of thiol groups and disulfide bonds of the thiomers. Exemplary results of the transport studies are shown in Fig. 1, P_{app} values are shown in Table 2. The permeation enhancing effect of thiolated PAH is very likely due to inhibition of the efflux pump P-gp, although the mechanism of P-gp inhibition by thiomers is still unclear. The introduction of thiol groups into the polymer leads to an improved permeation compared to the corresponding non modified polymer, therefore it is assumed that covalent interactions with cysteine residues on the cell surface are responsible for the effect. As the polymer cannot enter the cells, inhibition cannot be due to covalent binding to cytoplasmic cysteine residues, ATP-depletion, membrane fluidization or substrate competition. The inhibitory effect might occur via the formation of disulfide bonds with cysteine residues of the cell membrane, leading to changes of the membrane structure and allosteric changes in the protein's conformation. Furthermore, P-gp inhibition could be based on the polymer clogging the protein channel and thus sterically hindering drug binding and active efflux. The cross-linked polymer and thiomer had no significant efflux pump inhibitory effect in these experimental settings. The medium size thiomer (PAH-NAC 70 kDa) displayed the best improvement ratio of 1.77 in comparison to low size (PAH-NAC 15 kDa) 1.47 and crosslinked (cPAH-NAC) 1.10. It can be assumed that larger polymers cannot diffuse into the mucus gel layer and thus do not interact with the target structures on the cell membrane.

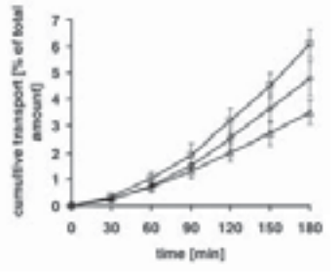

Fig. 1: Rho-123 permeation across rat small intestinal mucosa, in the presence of PAH 70 kDa (◇), PAH-NAC 70 kDa (□) and buffer only (△). Values are means ±SD of at least 3 experiments.

Table1: Amount of immobilized free thiol groups and disulfide bonds [µmol/g]

	Thiol groups	Disulfide bonds
PAH-NAC 15 kDa	77.6 ± 6.5	125.2 ± 25.3
PAH-NAC 70 kDa	83.1 ± 5.8	68.4 ± 13.0
cPAH-NAC	162.5 ± 11.2	93.3 ± 14.7

*Table 2: P_{app} values for Rho-123 across the intestinal mucosa of rats. Values are means ±SD of at least 3 experiments. *Differs from control (p<0.05)*

	P_{app} [cm/s]	SD [cm/s]	R
buffer only	$5.06*10^{-6}$	$6.50*10^{-7}$	1.00
PAH 15 kDa	$6.59*10^{-6}$*	$1.40*10^{-7}$	1.30
PAH 70 kDa	$6.91*10^{-6}$	$9.72*10^{-7}$	1.37
PAH-NAC 15	$7.43*10^{-6}$*	$1.67*10^{-7}$	1.47
PAH-NAC 70	8.9810^{-6}*	$5.45*10^{-7}$	1.77
cPAH	$5.00*10^{-6}$	$2.51*10^{-6}$	0.99
cPAH-NAC	$5.57*10^{-6}$	$3.56*10^{-6}$	1.10

CONCLUSION

Within this study, the permeation enhancing effect of a new thiomer – poly(allylamine hydrochloride)-*N*-acetylcysteine – was evaluated. It could be demonstrated that PAH-NAC conjugates improve the uptake of the model substrate rhodamine-123 most likely by inhibition of P-gp mediated efflux, showing best results for a medium molecular mass.

References

1. Takano, M. et al. Expression and function of efflux drug transporters in the intestine. Pharmacology & Therapeutics 109: 137-161 (2006)
2. Bernkop-Schnürch, A. et al. Thiomers. The Next Generation of Mucoadhesive Polymers. Am J Drug Deliv 3: 141-154- (2005)
3. Duggal, A. et al. Novel dosage forms and regimens for sevelamer-based phosphate binders. J.Ren Nutr. 16: 248-252 (2006)
4. Bernkop-Schnürch, A. et al. Development of controlled drug release systems based on thiolated polymers. J Control Release 66: 39-48 (2000)

PO054

Concentration and time dependent effect of poly-L-arginine on the permeability of pig urinary bladder wall

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INTRODUCTION

Severe urinary bladder infections or superficial bladder cancer can be treated by intravesical instillation of an appropriate drug. A local treatment lowers systemic toxicity of the applied drug and increases its concentration at the site of action. A cationic polysaccharide chitosan has already been proven to significantly increase the permeability of the urinary bladder wall (1) and when applied together with a drug it could improve the effectiveness of intravesical therapy. The main mechanism of enhanced permeability caused by chitosan is desquamation of urothelium, which covers the luminal surface of the bladder wall (1).

The aim of the present work was to test another cationic polymer poly-L-arginine for its ability to increase the permeability of pig urinary bladder wall.

METHODS

Permeability experiments were performed on isolated pig urinary bladders, obtained from a local slaughterhouse. All experiments were performed within 5 hours of sacrifice. The middle part of the urinary bladder was cut into four pieces (approximately 25x25 mm) and each piece was mounted into a diffusion cell, developed at the Faculty of Pharmacy, Ljubljana (2). In the first series of the experiments the luminal side of the bladder wall was exposed for 60 minutes to the solution of pipemidic acid only or to the solution of pipemidic acid with 0.0005, 0.005 or 0.05% (w/v) poly-L-arginine (molecular weight 70-150 kDa, Sigma-Aldrich, St. Louis, USA). In the second series of the experiments the tissue was exposed for 15, 30, 60 or 90 minutes to the solution of pipemidic acid with 0.005% (w/v) poly-L-arginine. All solutions were prepared in phosphate buffer (0.472 g Na_2HPO_4, 0.095 g KH_2PO_4 and 1.6 g NaCl per 1 L of deionised water) and pH of all solutions was adjusted to 4.5. In all the experiments the concentration of pipemidic acid (Lek, Ljubljana, Slovenia) was 0.014% (w/v). At the end of the experiments the tissue was washed with PB, rapidly frozen with liquid nitrogen and sectioned by cryostat (Leica CM 1850, Germany) in segments of 20 μm thickness parallel to luminal surface up to 1.2 mm of the tissue depth. After extraction of pipemidic acid from the tissue segments, drug concentration was determined by HPLC.

RESULTS AND DISCUSION

Urothelium belongs to tight epithelia and is normally impermeable to substances present in urine (3). Polymers that increase the bladder wall permeability could improve the effectiveness of intravesical therapy, but the permeation of toxic substances could also be enhanced. The effect of cationic polyamino acid poly-L-arginine on the urinary bladder wall permeability is shown in Fig. 1. 0.05 and 0.005% (w/v) polymer significantly increased the permeation of pipemidic acid into the bladder wall. With increasing tissue depth the effect of the polymer diminished. At 0.005% (w/v) concentration of poly-L-arginine the maximal effect on the tissue permeability seems to be approached and a further increase in the polymer concentration did not additionally enhance the permeability.

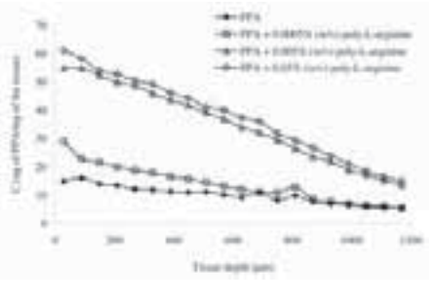

Figure 1: The amounts of pipemidic acid (PPA) that permeated into the urinary bladder wall in the 1st series of the experiments as a function of the tissue depth (mean ±S.D., n=6). The tissue was exposed for 1 hour to different concentrations of poly-L-arginine.

The results are in accordance with Tzan et al. (4) who ascertained that poly-L-arginine increased transepithelial conductance of rabbit urinary bladders. Moreover, poly-L-arginine increased the intranasal absorption of model drugs without nasal membrane damage (5).

Additionally, the time dependence of poly-L-arginine¢s effect on the permeation of the model drug into the bladder wall was ascertained. As seen from Fig. 2 the influence of poly-L-arginine on the tissue amounts of pipemidic acid gradually increased within 90 minutes.

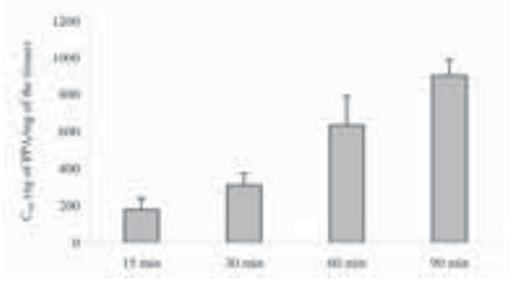

Figure 2: The cumulative amounts of pipemidic acid (PPA) permeated into the bladder wall in the 2nd series of the experiments. The tissue was exposed for different time periods to a solution of PPA with 0.005% (w/v) poly-L-arginine (mean ±SD, n=6).

To conclude, cationic polymers chitosan and poly-L-arginine are capable to significantly increase permeability of the urinary bladder wall. It seems their positive charge is important for interactions with negatively charged urothelial surface.

References

1. Kerec Kos M, Bogataj M, Veranič P, et al. Permeability of pig urinary bladder wall: time and concentration dependent effect of chitosan. *Biol Pharm Bull*, 2006, 29(8): 1685-1691.
2. Kerec M, Bogataj M, Veranič P, et al. Permeability of pig urinary bladder wall: the effect of chitosan and the role of calcium. *Eur J Pharm Sci,* 2005, 25 (1): 113-121.
3. Lewis SA. Everything you wanted to know about the bladder epithelium but were afraid to ask. *Am J Physiol Renal Physiol,* 2000, 278: 867-874.
4. Tzan CJ, Berg JR, Lewis SA. Modification of epithelial permeability by cationic polypeptides. *Am J Physiol*, 1993, 265: 1637-1647.
5. Ohtake K, Maeno T, Ueda H, et al. Poly-L-arginine predominantly increases the paracellular permeability of hydrophilic macromolecules across rabbit nasal epithelium in vitro. *Pharm Res*, 2003, 20(2): 153-160.

PO055

The research of the fluorquinolone – metal cation interactions

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INTRODUCTION

A clinically relevant interaction between a fluoroquinolone (FQ) and a metal cation was first described in 1985. Numerous clinical studies in which the authors investigated drug-drug interactions between FQ and preparations containing metal cations followed. Already the authors of the first report have speculated that the described drug-drug interaction might be related to the formation of complexes (coordination compounds) between the FQ and the metal cation, which cannot be absorbed [1]. This speculation was based on an earlier finding that a quinolone drug nalidixic acid forms coordination compounds with several metal cations. Interactions between FQ and preparations containing metal cations were studied in numerous clinical studies which were reviewed by Lomaestro and Bailie [3].

Although with the intention to explain the reduction of FQ bioavailability in the presence of metal cations, the bulk of scientific effort was directed into research of the chelation chemistry of FQ while only some attempts were made to explain the mechanism of the drug - metal cation interaction. Therefore, even recent literature most frequently describes the mechanism simply as "formation of nonabsorbable chelates", despite the fact that chelation has never been confirmed experimentally as a reason for the reduction of FQ bioavailability.

The purpose of this work is first to gather the attempts previously made to elucidate the biopharmaceutical mechanism of the FQ - metal cation interaction. Then some of the proposed mechanisms will be evaluated experimentally.

METHODS

The literature data was obtained by searching different combination of terms fluoroquionolones, quinolones, metal cations, interaction, bioavailability, mechanism etc. through PubMed and by tracing the cited references of the papers found through the PubMed searches.

The influence of metal cations on the solubility of FQ in a biologically relevant medium – bicarbonate buffer pH=7 was determined by the following procedure. An excess of FQ and 1.5 mL of the medium containing the metal cations were placed in pressure resistant plastic vials, which contained the volatile buffer component (carbon dioxide). Ion strength of all media was set to 0.1 by NaCl. The pH value was controlled before and after shaking at 120 rpm for 60 min (a preliminary experiment has shown that the equilibrium solubility is reached after 10 - 20 minutes) at 36°C. Afterwards, the samples were filtered through 0.22 μm pore size filters and diluted 100-fold for subsequent HPLC analysis.

The permeability of FQ (with or without metal cations) was measured through rat jejunum from male Wistar rats (250 – 320 g) in Easy Mount® side-by-side diffusion chambers as previously described [4]. The permeability of each FQ (5 mM donor solution) was evaluated in the

first phase (100 min) of the experiment. This first phase was followed by the second phase (also 100 min), during which the donor solution also contained a 5 mM concentration of the tested metal cation.

The stability of ciprofloxacin in the simulated gastric fluid was tested thermostated to 37°C over a three hour period.

RESULTS AND DISCUSSION

The literature review revealed several possible mechnisms of the FQ – metal cation interaction:

- altered FQ solubility,
- impaired chemical stability in the lumen,
- reduced permeability of FQ after complexation with metal cations,
- adsorption of FQ on precipitated metal cation hidroxydes and carbonates.

The most conflicting opinions can be found regarding the effect of metal cations on the solubility of FQ. Metal cations are reported to increase the solubility of FQ as well as to decrease it and thus cause the decreased bioavailability. Our own recent findings clearly demonstrate that the solubility of several FQ is either not affected or is even increased by metal cations (*Figure 1*).

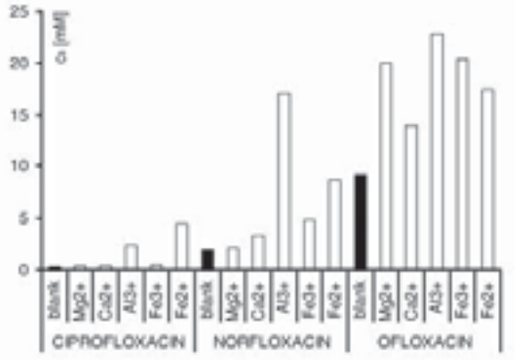

Figure 1: The influence of metal cations on the solubility of FQ

We have also tested the chemical stability of ciprofloxacin in simulated gastric fluid without and with the presence of Al^{3+} and Mg^{2+}, only to confirm that the FQ is perfectly stable regardless the presence of metal cations.

The effect of metal cations on the permeability of FQ was studied by different authors by determination of chloroform or octanol / water partition coefficients and by permeability measurements in different rat intestinal models. The partition coefficients were reduced by some (but not all) of the relevant metal cations and the rat intestinal permeability models in the "in vitro" setting sometimes correlated well with the "in vivo" drug-drug interaction. Our experiments with rat intestine in Sweetana-Grass type diffusion chambers showed that the interaction can only be observed "in vitro" if the donor solution is saturated by FQ and by metal cation (*Figure 2*). However, the reduction of FQ permeability is might not be sufficient to explain the very high clinically observed reductions of FQ bioavailability.

During a permeability study on a rat intestinal model Tanaka et al. have observed that precipitation in the donor solution took place. They have suggested that FQ might be adsorbed on the particles of aluminum hydroxide, which most probably precipitate in the duodenum and jejunum after ingestion of aluminum containing antacids. Such adsorption could significantly decrease the amount of the given dose prior to absorption [5].

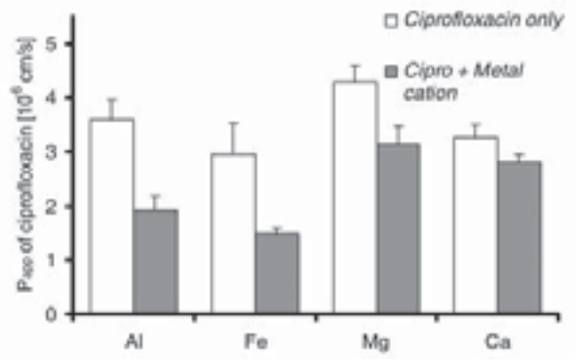

Figure 2: Permeability of saturated ciprofloxacin in the absence and presence of metal cations

CONCLUSION

We can conclude, that metal cations do not decrease the bioavailabilty of FQ by decreasing their solubility or chemical stability in the gut lumen. The "in vitro" FQ permeability is decreased in the presence of metal cations if the donor solution is saturated by FQ and by the metal cation salt, as it is in the “in vivo” situation. Unfortunately, the explanation with the adsorption of FQ on precipitates presumably formed in the gut will be very difficult to confirm in any kind of “in vitro” simulation.

References

1. Höffken G. et al., (1985) Reduced enteral absorption of ciprofloxacin in the presence of antacids. Eur J Clin Microbiol 4: 345.
2. Nakano M. et al., (1978) Interactions of Aluminium, Magnesium and Calcium Ions with Nalidixic Acid. Chem Pharm Bull 5: 1505.
3. Lomaestro BM and Bailie GR. Absorption interactions with fluoroquinolones. 1995 update, Drug Saf 1995; 12: 314-333.
4. Žakelj, S. et al., 2004. The influence of buffer composition on tissue integrity during permeability experiments "in vitro". Int. J. Pharm., 272, 173-180.
5. Tanaka M. et al., Mechanistic study of inhibition of levofloxacin absorption by aluminum hydroxide. Antimicrob Agents Chemother. 1993 Oct;37(10):2173-8.

PO056

Predictive capacity of immobilised artificial membrane chromatography in homogenous group of substances

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In the drug development and leading candidate selection investigation of the drug-membrane partitioning is a very critical step since it directly reflects the absorption of the drug from different biological barriers including small intestine or blood brain barrier. Several methods have been developed through out the past years for the prediction of drug membrane partitioning that would enable the fast screening of drug candidates. One of these methods is the immobilized artificial membrane (IAM) chromatography. IAMs are monolayers of phospholipids that are bound to silica particle to mimic the lipid environment in the cell membrane. Using this methods the substances are characterized in terms of permeability based on their retention on these stationary phases. It has been showed that retention factor kIAM is well correlated with the permeability from the substances when used in a heterogeneous group of substances from different permeability classes. It is more important to determine the permeability differences for the drug candidate selection in a homogenous group, thus in this work the relationship between kIAM, Caco-2 permeability and log P is investigated in a homogenous group of 16 steroidal substances utilizing both isocratic and gradient methods for the determination of kIAM values. The Caco-2 permeability values are also further correlated with thermodynamic parameters such as enthalpy and entropy of the partitioning in to the IAM column determined in 3 different temperatures of 27, 37 and 47 °C. It has been showed that correlation of kIAM extrapolation values with log P and Papp values of the substances tested is better compared to the correlations with kIAM gradient values with correlation coefficients of r2 = 0.92 and 0.48 respectively. Furthermore the best correlation with the thermodynamic parameters are achieved with the correlation of Papp and entropy (Δ S) with a correlation coefficient of r2= 0.55. As a results it is concluded that kIAM values gathered with extrapolation method is more predictive due to the better differentiation for the homogenous group of substances. On the other hand IAM shows the same trend with the log P permeability correlation where from a certain point increase in the kIAM reflected as the decrease in the permeability.

PO057

Permeation and enhancement properties of sodium-deoxycholate gels containing betamethasone-17-valerate

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INTRODUCTION

Topical corticosteroids are the most frequently used drugs in dermatological practice currently. Despite their demonstrated effectiveness as treatment for psoriasis or atopic dermatitis, topical corticosteroids are associated with various side effects that may limit their use [1]. One of the methods to reduce the adverse effects of these drugs is to enhance the permeability of corticosteroids so as to reduce the topically applied dose [2]. Betamethasone-17-Valerate (BMV) which is a potent topical steroidal drug was selected as a model drug in this study. Sodium-deoxycholate (Na-DOC), a naturally occurring bile salt is a low molecular weight substance which is able to form gels when in contact with excess buffer systems. Na-DOC gels are multifunctional drug carrier systems which also act as penetration enhancers [3]. The aim of this study is to evaluate the permeation properties of BMV from Na-DOC gels and compare with the commercial cream formulation to investigate the permeation enhancement effect.

EXPERIMENTAL

Materials

BMV was kindly gifted from GlaxoSmithKline. Na-DOC was purchased from Fluka and Mannitol was provided from Merck. All other chemicals were of analytical grade.

Preparation of Gel Formulations

Na-DOC (0.5 %) was dissolved in phosphate buffer saline (PBS). PBS consisted of phosphate buffer (pH=7.2) and 0.9 % sodium chloride. Mannitol (5 %) was added to this solution. Finally, BMV was incorporated to the formulations at two different concentrations (0.05 and 0.1

%). Commercial cream formulation, used for comparison was containing 0.1 % BMV.

***In Vitro* Permeation Studies**

The *in vitro* permeation experiments were performed by using Franz diffusion cells through rat abdominal skin which was placed in continuously stirred (600 rpm) ethyl alcohol-distilled water (1:1) mixture at 37 ± 0,5°C. The available diffusion area between cells was 0,64 cm^2 and the volume of receptor phase was 5 ml. After serial sampling at specified time intervals, the amount of BMV was determined by HPLC at 240 nm. Acetonitrile/water (60:40) was used as a mobile phase with a flow rate of 1 ml/min. The cumulative amount of BMV was calculated and plotted as a function of time. The slope of the linear portion of the plot was calculated as he steady state flux (mg/cm^2/h). Each data point represents the average determination of three experiments.

Determination of Enhancement Efficacy

The permeation enhancing activities were expressed as enhancement ratios of flux (ER_{flux}).

$$ERflux = \frac{\text{BMV flux in Na-DOC gels}}{\text{BMV flux in commercial cream}}$$

RESULTS AND DISCUSSION

The permeation experiments were performed using freshly excised rat abdominal skin as barrier. Rat abdominal skin has been shown to be a reasonable model for human skin *in vitro* in passive conditions [4].

The release profiles of BMV from formulations were given in Figure 1. At the end of 8h, Na-DOC gels were showed significantly higher drug release when compared to commercial cream formulation at both concentrations (Figure 1).

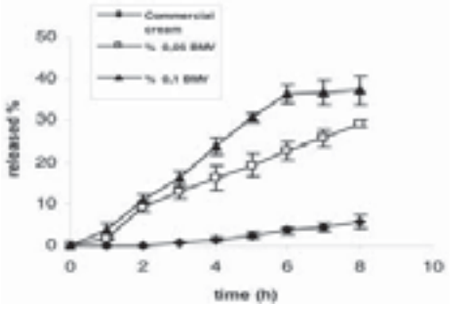

Figure 1: The release profiles of BMV through rat abdominal skin

The permeation parameters of BMV across rat abdominal skin were shown in Table 1. The effect of formulation type on the flux of BMV was also shown in Figure 2.

When the flux data evaluated together, the flux of BMV from Na-DOC gels was about 2,51 and 8,47 times higher compared with commercial gels. In addition, the permeated amount of drug at the end of six hours was significantly higher in Na-DOC gel formulations (Table 1 and Figure 2).

By the way, when the drug concentration reduced 2 times in Na-DOC gel, amount of permeated BMV and calculated flux value were 2,97 and 2,51 times higher than commercial cream, respectively (Table 1).

Table 1: Permeation parameters of BMV across rat abdominal skin

Formulation	Amount of drug released for 6h (mg/cm^2)	Flux (mg/cm^2/h)	ER_{flux}
Commercial cream	0,00375 ± 0,0003	0,00077 ± 0,0001	1,0
0,05 % BMV	0,011019 ± 0,0013	0,00193 ± 0,0001	2,51
0,1 % BMV	0,036094 ± 0,0006	0,006525 ± 0,0003	8,47

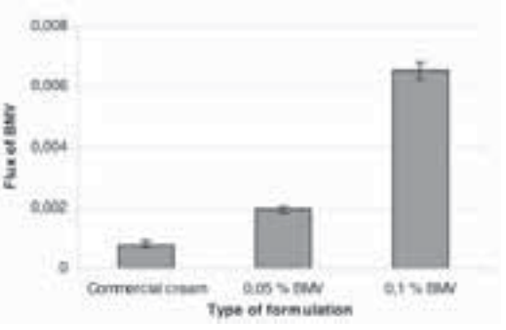

Figure 2: Effect of formulation type on the flux of BMV

These results indicate that Na-DOC was significantly increased the skin permeability of BMV.

CONCLUSION

The percutaneous absorption of BMV was investigated from Na-DOC gel formulations. It was seen that even the drug concentration was reduced two times in Na-DOC gels, the amount of permeated drug was nearly 3 times higher than commercial cream. Na-DOC gels were significantly enhanced the percutaneous absorption of BMV when compared to commercial cream formulation. Therefore, Na-DOC gel formulations could be suggested as promising vehicles for BMV as well as other topical steroidal drugs for reducing adverse effects depending drug dose.

ACKNOWLEDGEMENTS

The authors are grateful to the Novartis Drug Company for its financial support of this study.

REFERENCES

1. Del Rosso J. Friedlander S.H. Corticosteroids: Options in the era of steroid-sparing therapy. J. Am. Acad. Dermatol. 53(1) 50-58 (2005).
2. Fang J.Y. Fang C.L. Sung K.C. Chen H.Y. Effect of low frequency ultrasound on the in vitro percutaneous absorption of clobetasol-17-propionate. Int. J. Pharm. 191 33-42 (1999).
3. Valenta C. Nowack E. Bernkop-Schnürch A. Deoxycholate-hydrogels: novel drug carrier systems for topical use. Int. J. Pharm. 185 103-111 (1999).
4. Godin B. Touitou E. Transdermal skin delivery: Predictions for humans from *in vivo, ex vivo* and animal models. Adv. Drug Deliv. Rev. 59 1152-1161 (2007).

PO145

Poloxamer influence on rheology, in vitro release and permeability of 1,7-heptanedicarboxylic acid formulations

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INTRODUCTION

1,7-heptanedicarboxylic acid (HDA) is used for topical treatment of acne and inflammatory papules and pustules of mild to moderate rosacea [1]. It is naturally occurring saturated dicarboxylic acid poorly soluble in water. Its pKa_1 is 4.53 and pKa_2 is 5.33 [2], logP is 1.646.

Different hydrogel-thickened o/w emulsions intended for skin delivery of HDA were investigated. Vehicles have been prepared of medium-chain triglycerides used as the oil phase, mixture of soybean lecithin and non-ionic surfactants as the emulsifiers. Drug was supersaturated in the vehicle.

Non-ionic surfactants (and lecithin) were used to emulsify oil/water mixture. It was noticed that surfactants may affect physical properties of the vehicle, which potentially could change drug diffusion from the formulation (Fick s law) [3].

The aim of this study was to investigate influence of different surfactants on vehicle properties and their impact on drug in vitro release and permeation profiles in order to define rate-limiting step of particular drug delivery system.

EXPERIMENTAL METHODS

Materials

Medium-chain triglyceride was provided by Sasol, Germany. Poloxamers 188 and 407 were purchased from BASF, Germany and soybean lecithin from Lipoid, Germany. Propylene glycol was obtained from Dow, Germany. Polyacrylic acid was obtained from Lubrizol, USA. Sodium hydroxide was provided by Kemika, Croatia. HDA was obtained from CU Chemie Uetikon GmbH.

Preparation of hydrogel-thickened o/w emulsion

Oil component has been emulsified with mixture of propylene glycol, soybean lecithin and different poloxamers using homogenizer. Emulsions have been thickened with crosslinked polyacrylic acid polymers. HDA has been added into vehicle and pH adjusted to 4.8. Poloxamer types and quantities examined in the study are listed in Table 1.

Table 1: Poloxamer types and quantities.

Formulation	Poloxamer	Quantity (%, w/w)
A	188	0.5
B	188	1.5
C	407	0.5
D	407	1.5

Rheological studies

Rheological testing of the various formulations was performed at 25°C using rheometer PHYSICA MCR 301, Anton Paar and 5 cm parallel plate with 1 mm gap. A flow curve was determined with a following parameters: a continuous ramp with a shear rate as controlled variable (0,1-72s^{-1}), log mode, 1 minute preshearing (2 s^{-1}).

In vitro release studies

Franz diffusion cells were mounted with artificial membranes (RC; regenerated cellulose, 0.2 μm pore size). The receptor media contained USP acetate buffer pH 5.5 thermostated at 35°C. Samples were withdrawn at specific time intervals during 7.5 h and analyzed.

In vitro skin permeation studies

Full thickness pig skin has been used. Hair was removed by shaving, subcutaneous and adipose tissue carefully removed and presoaked in phosphate buffer saline (PBS) of pH 7.4.

Skin has been mounted on Franz diffusion cell. The receptor media contained PBS, pH 7.4, thermostated at 37°C. The formulation (1 mL) was applied on the skin surface and covered to prevent evaporation. Samples were withdrawn at specific time intervals and analyzed. In order to determine the amount of drug retained in the skin after 24 h, the skin was rinsed off with tap water and homogenized with a methanol:PBS 1:1 mixture.

RESULTS AND DISCUSSION

Rheological studies revealed the difference in viscosity between different types of poloxamers used regardless of their quantity (Figure 1.). Poloxamer 188 generated products of the higher viscosity in comparison to poloxamer 407. The flow curves of all tested formulations show significant viscosity decrease with increasing shear rate, indicating easy modulated structure with a shear-thinning behavior.

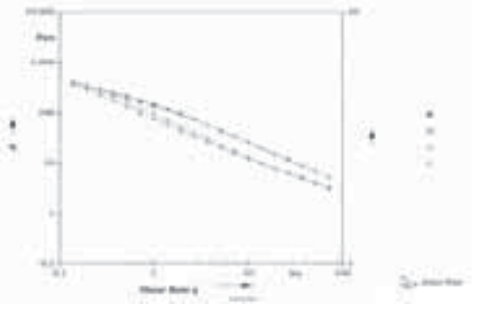

Figure 1: The flow curves of tested formulations.

Ability of a formulation to release a drug is important aspect of formulation development. Data obtained from in vitro release studies (Figure 2.) have shown that release rate is also impacted by poloxamer type selected.

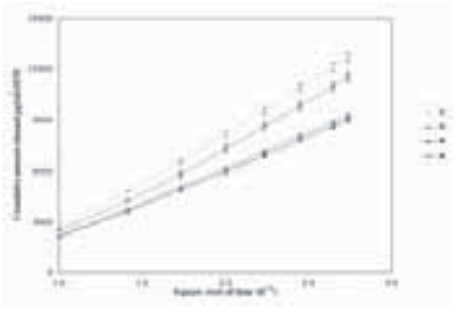

Figure 2: Drug release profile comparison of different formulations.

Statistical analysis confirmed a significant difference (90% confidence interval) between release rates of formulations containing poloxamer 188 with regard to formulations containing poloxamer 407. Results confirmed that increase in formulation viscosity attributes to decrease of drug release.

Since rate-controlling step for drug delivery into skin could be its release from the vehicle or its permeation through outermost layer of the skin – in vitro permeability studies on the skin were conducted for selected samples. The amount of drug permeated through the skin is two-fold higher for the less viscous formulation while the amount of drug retained in the skin is similar between tested formulations (Table 2.).

Table 2: Drug permeated and retained in the skin after 24 h.

.	[µg/cm^2 ±SEM] penetrated through skin	[µg/cm^2 ±SEM] retained in the skin
Formulation B	437.4 23.9 (n=3)	364.1 49.3 (n=3)
Formulation D	905.6 58.6 (n=6)	421.7 101.5 (n=3)

CONCLUSION

Poloxamers may influence the viscosity properties of the vehicle attributing to release characteristics of drug. Results suggest that rate-limiting step for examined formulations is drug release from the vehicle.

References

1. D. Thiboutot, New treatments and therapeutic strategies for acne, Arch. Farm. Med., 9 (2000), 179-187.
2. Merck Index on CD-ROM, Version 12:3
3. K. A. Walters, Dermatological and Transdermal Formulations, in: A.A. Davis, R. J. Gyurik, J. Hadgraft, M. A. Pellet, K. A. Walters, Formulation Strategies for Modulating Skin Permeation, Marcell Deker Inc., New York, 2002, 271-399.

PO059

Preliminary study on bioavailability of self-emulsyfied oxytetracycline hydrocloride in rainbow trout after single and multiple oral administration

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INTRODUCTION

Oxytetracycline HCl is a broad-spectrum antibiotic, widely used in aquaculture to treat systemic bacterial infections. Many pharmacokinetic and bioavailability studies have been carried out on OTC [1-3]. The apparent oral bioavailability of OTC has been reported to be very low in rainbow trout: 1.25%-5.6% [4]. To improve oral absorption of OTC in fish, some researches were already undertaken. The different approaches were aimed to formulate the drug including it in different systems, like microencapsulation in etheroglycosides [5]. The objective of this work was to develop a new medicated feed formulation, containing the drug in a self-emulsifying drug delivery system (SEDDS). SEDDS are isotropic mixtures of oil, surfactant and drug, that form fine oil/water microemulsion when in contact with physiological fluids [6]. The developed formulation was assessed in rainbow trout after administration with a gastric probe and after single and repeated administrations of medicated feed freshly prepared with SEDDS.

EXPERIMENTAL METHODS

Preparation of SEDDS formulation:

OTC E.P. grade (Galeno) was incorporated in the oily phase (Miglyol, Caelo). The resulting solution was mixed with the surfactant polysorbate 80 (Tween 80®, Sigma Aldrich) in appropriate proportions, under gentle stirring with a magnetic stirrer for 30 min.

Then, the medicated feed was prepared at Hendrix S.p.A. by mixing the classical non medicated feed for trout (composed of fish flour, wheat gluten, wheat starch, vitamin and mineral supplements and soy lecithin) with SEDDS (40% w/w).

The developed formulation was then assessed in rainbow trout after administrations with a gastric probe in comparison to an aqueous solution (trial 1) or supplied directly in the tank water and compared to the commercial medicated feed (trial 2) as single and repeated administrations.

***In vivo* trial 1:** One hundred and sixty rainbow trout were randomly divided in two tanks (80fish/tank) and they were individually supplied 1 ml of aqueous solution of OTC (12 mg/ml) to one group and 1 ml of SEDDS formulation (12 mg/ml) to the other. The two different formulations were administered with a gastric probe after sedation with tricainemethansulfonate (MS-222, Syndel Laboratories Ltd.). At scheduled times plasma samples were withdrawn from caudal vein of ten fish for each time point and then stored at -20°C until HPLC analysis was performed, After solid-phase extraction.

***In vivo* trial 2:** Three hundred and sixty rainbow trout were used to compared commercial medicated feed with freshly prepared medicated feed containing SEDDS. Medicated feeds, both containing 7,5g/kg of OTC were supplied to fish (1% w/w) as single and repeated administrations (for 5 days).

Blood samples were collected at scheduled times (single dose: 1-4-6-8-10-12h; repeated doses: 24-36-48-60-72-84-96-108-120-132-144-156h) and plasma was analysed following the previously described procedure.

RESULTS AND DISCUSSION

Trial 1: The results showed an AUC value of 78,29 (μg h/ml) for aqueous solution, and of 423,83 (μg h/ml) for SEDDS. The bioavailability of SEDDS was 5,41 times higher than the aqueous solution one, as depicted in Fig. 1.

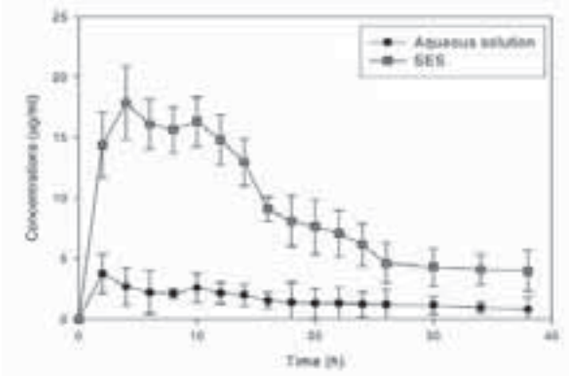

Figure 1: Plasma profiles obtained after administration with gastric probe.

Trial 2: After repeated administrations the following AUC values were observed: 92,09 (μg h/ml) and 376,48 (μg h/ml) respectively for commercial medicated feed and for freshly prepared medicated feed containing SEDDS. The second one therefore resulted to be 4,09 times more bioavailable (Fig. 2a and b).

It can be concluded that these systems represent a great advantage because they enhance significantly the oral bioavailability of OTC, thus giving the possibility to obtain the same effect in the body with a much lower dose of drug. This is very important in aquaculture field because it reduces the environmental pollution and the outcome of antibiotic resistance phenomena in trout. Hence upon these considerations many hopes account the possibility of further extending the work on different fish species.

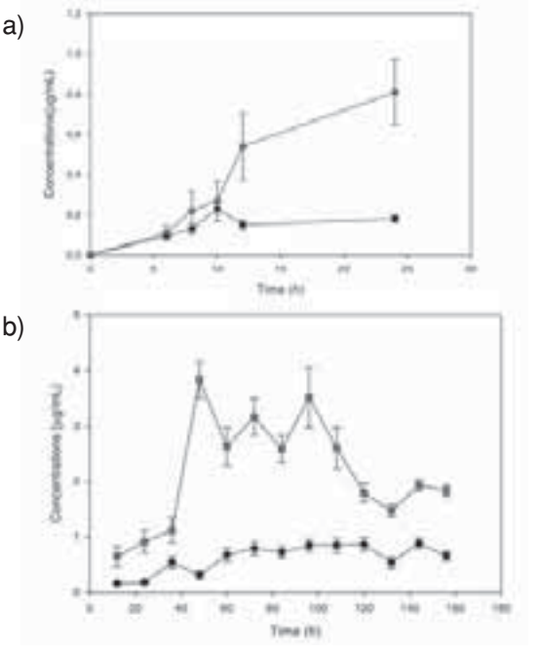

Figure 2: Plasma profiles after single administration (a) and repeated administrations (b) of medicated feeds [black symbols= commercial; red symbols=SEDDS .

References

1. Grondel et al., *J. Vet. Pharmacol. Ther.* 12, 157–162 (1989).
2. Bjòrklund, *Xenobiotica* 21, 1511–1520 (1991).
3. Black et al., *J. vet. Pharmacol. Therap.*, 14, 351-358 (1991).
4. Nouws et al., In: *Pharmacokinetics of Antimicrobials in Some Fresh Water Fish Species*, Off. Int. Epiz., Paris, pp. 437–447 (1992).
5. della Rocca et al., Ittiopatologia (2005),2, 137-144.
6. Shah et al., 1996 Int. J. Pharm., 106, 15-23.

PO060

Towards an in vitro simulation of drug/drug interactions: ciprofloxacin/iron case study

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INTRODUCTION

Ciprofloxacin interaction with the concomitantly administered iron preparations is well known and described in the relevant literature [1-4]. The observed drug absorption impairment in the presence of iron is, generally, attributed to the formation of less absorbable complex adduct. However, literature data related to ciprofloxacin complexation and solubility in the presence of iron and/or other metal ions are somewhat contradictory. While ciprofloxacin tablet dissolution has been shown to be retarded, results of the solubility studies report no effect or increased drug solubility in the presence of divalent or trivalent cations [5-8]. In the present work, ciprofloxacin/iron interaction was simulated in vitro by performing the solubility and dissolution studies in reactive media in order to elucidate the potential interaction mechanism and propose dissolution methodology that would be indicative of the situation encountered in vivo.

EXPERIMENTAL

• In vivo data

Literature in vivo data on ciprofloxacin co-administration with ferrous sulphate tablets reported by Lehto et al [3] and intravenous ciprofloxacin administration [9] were evaluated. Ciprofloxacin absorption profiles were calculated by numerical deconvolution using data on ciprofloxacin intravenous administration as a reference (i.e. weighting function). For the in vitro in vivo correlation purposes, the obtained absorption profiles were normalized by the total fraction of drug absorbed.

• Dissolution study

Dissolution studies of commercially available ciprofloxacin tablets (Ciprobay 500, Bayer Schering Pharma) were performed under the compendially recommended conditions (water as the dissolution medium, paddle rotating speed 50 rpm) without and with different amounts of ferrous sulphate (FeSO4x7H20) added, using both the standard rotating paddle apparatus with 900 ml dissolution media, as well as the mini-paddle assembly with 250 ml dissolution media (Erweka DT 700). Dissolution media samples were withdrawn at the predetermined time intervals, appropriately diluted and assayed UV spectrophotometrically at 276 nm.

• Solubility study

Solubility studies in water and reactive media containing 2mg/ml and 10 mg/ml FeSO4x7H20, i.e. 7.2 mM and 36 mM Fe(II), respectively, were performed using both ciprofloxacin hydrochloride and/or ciprofloxacin base. The investigated samples were continuously shaken on a laboratory shaker (Unimax 1010, Heidolph) for three hours, and, after centrifugation (where necessary) and filtration, appropriately diluted and assayed UV spectrophoto-metrically at 276 nm.

RESULTS AND DISCUSSION

In all the interaction studies, instantaneous formation of yellow color upon contact of ciprofloxacin and FeSO4 occurred. Ciprofloxacin hydrochloride solubility in media containing 7.2 mM Fe(II), was equal to that in the pure water, nevertheless of the yellow coloration observed. However, in media containing 36 mM Fe(II), drug solubility was reduced to 23.7 mg/ml (~60 mM). Also, addition of FeSO4 to ciprofloxacin hydrochloride solution resulted in notable precipitation. In contrast, solubility of ciprofloxacin base was increased in the presence of FeSO4. Aqueous solubility of ciprofloxacin was determined to be 0.1 mg/ml (0.3 mM). In media containing FeSO4, drug solubility increased to the extent that no saturation was accomplished with ciprofloxacin levels up to 5 mg/ml (~14 mM). Although such data are in accordance with those reported by other authors, their in vivo relevance is questionable since the therapeutically relevant form of ciprofloxacin is its hydrochloride salt. Furthermore, the results obtained indicate that interference of the counterion present in reactive media should not be neglected.

The results of ciprofloxacin tablet dissolution studies in water with/without addition of ferrous sulphate are presented in Fig.1. The effect of drug-drug interaction was more pronounced in the mini-paddle assembly, where the concentration of both drugs was more than three times higher than in the standard apparatus. The reduction of the total amount of ciprofloxacin dissolved was in the rank order with the amount of ferrous sulphate added.

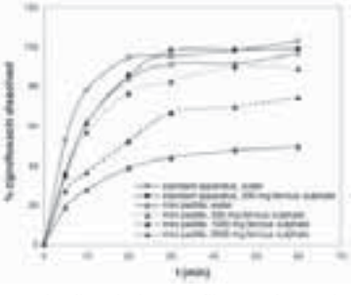

Figure 1: Ciprofloxacin dissolution profiles under various experimental conditions

• In vitro-in vivo correlation

The in vivo relevance of the dissolution studies performed was tested by comparing the obtained in vitro dissolution data with the deconvo-

luted in vivo data. Considering the difference in the time scale of the relevant processes encountered in vitro and in vivo, the in vitro time scale was extended by time scaling factor, TS=6. In order to provide sufficient data points for correlation purposes, scaled in vitro data were interpolated at dt=15 min time intervals. The best in vitro – in vivo correlation, defined by the slope of the regression line 1.167 and coefficient of correlation 0.980, was obtained for the set of dissolution data obtained in the mini paddle apparatus in water/media containing 10 mg/ml FeSO4x7H20. Corresponding in vitro – in vivo correlation plot is given in Fig. 2. Although the solubility of ciprofloxacin base was increased in the presence of ferrous sulphate, the results obtained showed that solubility of ciprofloxacin hydrochloride salt, under the same experimental setting, was reduced. Such data are in accordance with the reduced ciprofloxacin bioavailability when co-administered with iron, as well as the limited dissolution of ciprofloxacin tablets encountered in vitro in the presence of iron.

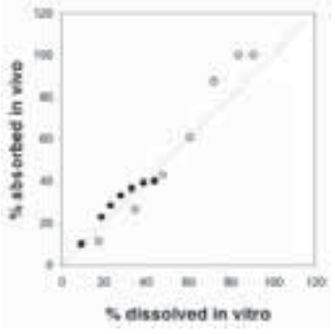

Figure 2: IVIVC plot

References

1. Campbell & Hasinoff, Br J Clin Pharmac. 31: 251-255, 1991;
2. Kara et al, Br J Clin Pharmac. 31: 257-261, 1991;
3. Lehto et al. Br J Clin Pharmac 37:82-85, 1994
4. Polk et al, Antimicrob. Agents Chemother. 33: 1841, 1989
5. akelj et al, Pharmazie. 62: 318-320, 2007
6. Sanchez et al. Int J Pharm 106: 229-235, 1994
7. Kozjek et al. Acta Pharm 46: 109-114, 1996
8. Sultana et al, Pak.J.Pharm.Sci. 18:23-31, 2005
9. Ljungberg & Nilsson-Erhle, J. Antimicrob. Chemother. 22: 715-720, 1988

PO061

Deciphering nifedipine absorption from modified release tablets

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INTRODUCTION

Drug absorption is a complex process affected by a number of factors related to drug substance properties, dosage form characteristics as well as the conditions encountered in vivo after drug administration. Identification of drug absorption or dissolution profile (i.e. in vivo input) is an important factor in drug product development and often a prerequisite in the course of in vitro - in vivo correlation development. Depending of drug disposition characteristics and type of in vivo data available, different pharmacokinetic model types may be used to assess drug input kinetics from the plasma concentration time profiles.
In the present study, comparative evaluation of the results obtained by applying the Loo-Riegelman absorption method (L-R) and numerical deconvolution (DECON) model to the same data set has been performed in order to determine nifedipine input kinetics from modified release tablets and evaluate the usefulness and applicability of each method.

EXPERIMENTAL

Loo-Riegelman method

Literature data on the food effects on two nifedipine modified release tablets reported by Schug et al [1] have been evaluated. In vivo data observed by Rashid et al [2] after the intravenous nifedipine administration were used to estimate drug disposition parameters. Biexponential curve fitting of the iv data was performed using MicrocalOrigin 5.0 (Microcal Software, Inc).

Numerical deconvolution

The same in vivo data set taken from the literature was evaluated. Plasma concentration profiles observed after intravenous and oral administration of immediate release capsule reported by Rashid et al [2] have been used as the weighting functions in order to estimate nifedipine input kinetics from the modified release tablets. Deconvolution was performed using an *in-house* software PharmPred based on the point-area method with staircase input function. Considering the sensitivity of deconvolution procedure to the time step size, in vivo profiles were interpolated at the constant and equidistant time intervals dt = 15 minutes using the Data Analysis Tool in MicrocalOrigin 5.0 (Microcal Software, Inc).

RESULTS AND DISCUSSION

For comparison purposes, the results obtained by L-R and DECON are presented individually for each of the investigated nifedipine modified release products under the fasted and fed-state study conditions (Figure 1 and 2). In the respective figures, solid lines refer to oral capsule plasma concentrations used as a weighting function, while the dotted lines represent the profiles calculated using intravenous data as the weighting function in the deconvolution algorithm.

The results obtained indicate that the absorption profiles calculated by numerical deconvolution are in a good agreement with data obtained by L-R. Although certain differences between the corresponding input profiles calculated by L-R and DECON are obvious, they generally follow

the same pattern. The estimated extent of nifedipine absorption is in accordance with the literature data on nifedipine bioavailability being reported in the range 45-56% owing to the first-pass effect (nevertheless of the complete drug absorption) [3]. In both the LR and DECON estimated profiles there was a difference of 15 to 20 % between the data calculated referring to the oral administration of rapidly available capsule and data calculated using intravenous profile as a reference. Lower estimates of the in vivo input calculated using intravenous reference, compared to oral capsule plasma profile used as a reference, could be reflective of the 'presystemic metabolism' which was reported for nifedipine.

In spite of the different underlying assumptions related to each methodology, the results obtained by Loo-Riegelman method and/or numerical deconvolution were in a good agreement with regards to both the rate and extent of nifedipine absorption. However, the input kinetics estimated using plasma profile observed in vivo after the administration of immediate release nifedipine capsule as a reference should be indicative of the in vivo drug dissolution. Therefore, it should be more advantageous as the targeted profile in the course of in vitro – in vivo correlation development and design of the 'biorelevant' dissolution methodology.

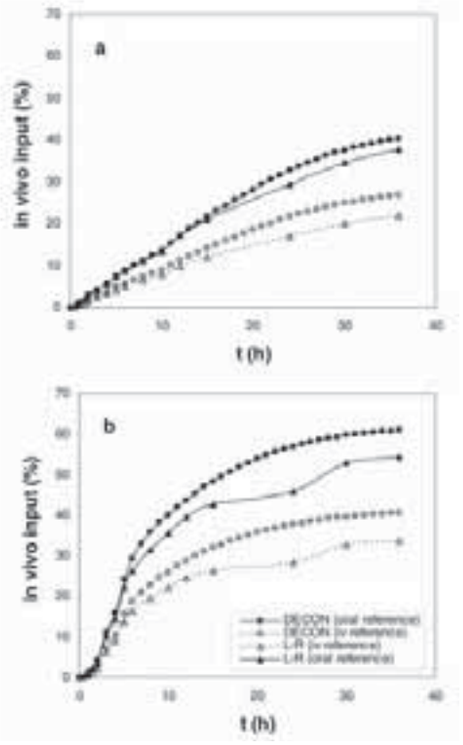

Figure 1: Nifedipine in vivo input profiles calculated by different methods for test product A: (a) fasted, (b) fed state

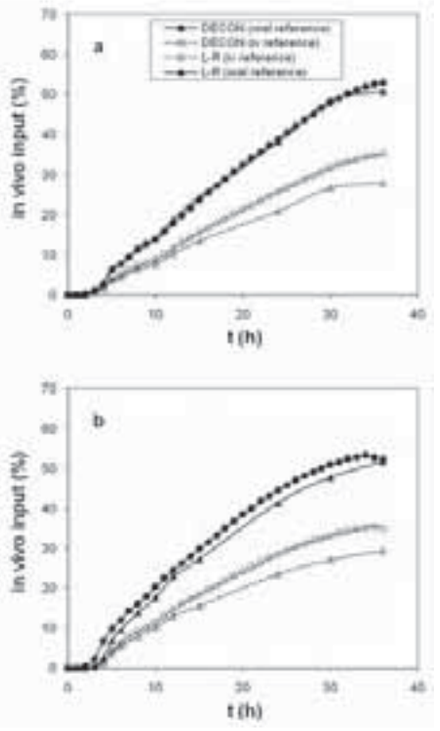

Figure 2: Nifedipine in vivo input profiles calculated by different methods for test product B: (a) fasted, (b) fed state

References

1. Schug et al, Eur J Pharm Sci 15: 279-285, 2002
2. Rashid et al, Br J Clin Pharmac 40: 51-58, 1995
3. Foster et al, J Clin Pharmacol 23: 161-170, 1983

PO062

High-energy mechanochemical activation of herbal drugs: the case of silybum marianum

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INTRODUCTION

Standardized extracts from the fruit seeds of *Silybum marianum* L. Gaertn. (milk thistle, Asteraceae), are used in humans for the treatment of liver diseases of different etiologies. The group of active constituents, named silymarin, mainly consists of silybin (40-60%) and isosilybin (10-20%) (each as pair of diastereoisomers A and B), silydianin and silychristin (20-45%) [1].

The therapeutic use of these flavonolignans is partly restricted by their insolubility in water. In particular, silybin, the main component, is sparingly soluble in water and spontaneously tends to form non-absorbable microcrystals. The oral bioavailability of this herbal medicine is therefore limited and it is very dependent on the galenical preparation, as shown for various silymarin products on the market [2].

Several approaches to improve the oral bioavailability of silymarin have been attempted, such as, for example, its complexation with phosphatidylcholine, solid dispersions with hydrophilic polymers and the formulation of a self-microemulsifying systems [3].

Whit respect to these experiences, the purpose of the present research is to ameliorate the bioavalability through a solid state mechanochemical activation process. In particular, the performance of two types of mills (a planetary and a vibrational one) were compared by processing different binary mixtures of dry extract with β-cyclodextrins (βCD). After the solid-state characterization of the coground systems in comparison to the simple physical mixtures, their *in vitro* dissolution and *in vivo* bioavailability were evaluated in comparison to a commercial formulation, containing the same dose of Sylimarin (Silirex® 200).

EXPERIMENTAL METHODS

Preparation and characterisation of coground systems

Silybum marianum dry extract (Indena, Milano) and β-cyclodextrins (BCD, Wacker Chemie, Germany) were processed in different weigth proportions in a planetary mill (Fritsch P7, P5, Pulverisette, Milano) or in a vibrational mill (Sweco M18/5, Florence, USA) using different velocities/frequencies and for different times. The coground were then characterised from the physico-chemical and dissolution point of view, and, their oral bioavalability in Whistar rats after administration by gavage was assessed.

RESULTS AND DISCUSSION

The characterisation of the coground mixtures (by PXRD and Hg-Porosimetry) revealed their amorphous character (Fig.1) and a remarkable enhancement of powders' surface area together with a significant reduction of particle size (Fig.2).

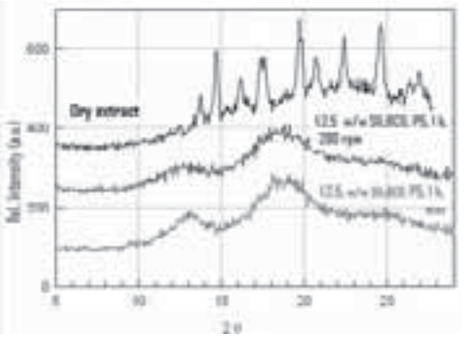

Figure 1: X-ray diffraction patterns

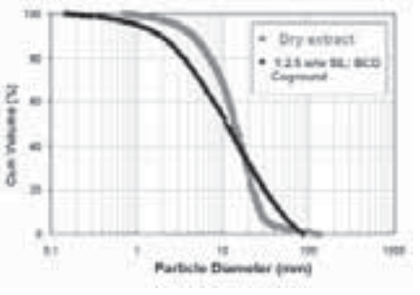

Figure 2: Hg porosimetry of a coground sample in comparison to the dry extract

The presence of an activated status of the dry extract was confirmed by the *in vitro* dissolution profiles that resulted dramatically ameliorated in comparison not only to the to dry extract alone, but also to the commercial formulation (Silirex® 200) and to the corresponding physical mixture. Further, the results pointed out the importance of the type of mill used for the cogrinding process and of the drug-to-carrier ratio. The best results were obtained from 1:2.5 w/w active-to-βCD coground prepared in planetary mill P5 for 1 h using the maximal velocity (Fig. 3).
The oral bioavailability of this formulation was finally evaluated in Whistar rats in comparison to a commercial formulation, containing the same dose of Sylimarin (Silirex® 200). These *in vivo* studies revealed that the *in vitro* dissolution enhancement corresponded to an effective *in vivo* oral bioavailability improvement of more than 6 times with respect to the commercial formulation. (see Table 1, and Fig. 4).

Table 1: Pharmacokinetic data

	C_{max} (mg/l)	AUC (mgh /l)	REL. BIOAVAIL.
Silirex® 200	4.1	16.2	1
1:2.5 w/w SIL:BCD cogr.	33.2	107.8	6.6

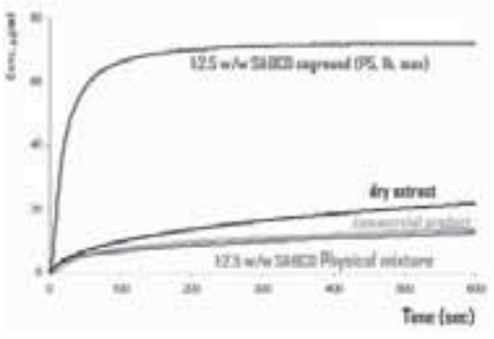

Figure 3: Comparison of the vitro dissolution profiles in deionized water using the paddle method.

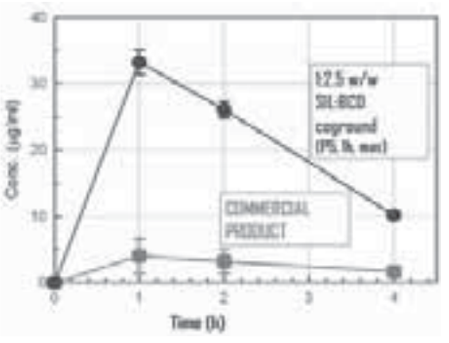

Figure 4: Plasma profiles after oral administration of the coground system and the commercial formulation.

References

1. ESCOP (European Scientific Cooperative on Phytotherapy), *Cardui Mariae Fructus*/Milk Thistle Fruit. January 2005.
2. Bulles H. et al., Arzneim-Forsch/ Drug Res 45, 61-64 (1995).
3. Wu W. et al. Eur J Pharm Biopharm 63, 288-294 (2006).

PO063

The study of the organism response to the administration of metoprolol tablets using chaos theory elements

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INTRODUCTION

The human organism can be considered as a mathematical model of a dynamic nonlinear system, irreversible in time, composed of several interconnected elements performing certain functions dictated by the internal structure, their state and by the values of external stimulae. Its behaviour can be represented as a signoid curve (fig. 1), with two thresholds: an inferior one (it must have a certain value, in order to allow mathematical processing) and a superior one (over a certain value, the system becomes saturated and does not respond to external stimulae). The irreversible characteristic of the system means that if its state is known at a certain moment in time, its state at a later moment can be deduced, but the deduction of the value which has lead to a certain response is not always possible.

Furthermore, during the administration of the drug, several variables, more or less random, intervene: the variation of the moment of oral administration, changes in diet, in rhytm and intensity of the physical effort, stress, etc. Thus, the reaction of the organism is variable in time.

Based on these considerations, we have tried to observe the variability of the tablets' effect on the human organism using chaos theory elements.[1, 2] Metoprolol is administered on a long term, thus allowing us to study the ograinsm's response over a long period of time.

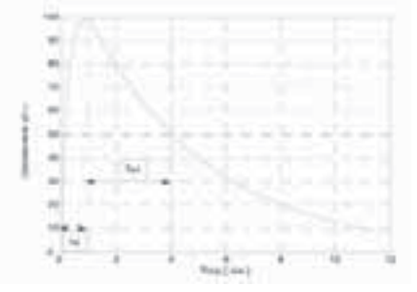

Figure 1: The variation of the plasmatic concentrantion for metoprolol: T_a=1,5-4 hours; T_{e1}=3-7 hours

EXPERIMENTAL METHODS

In the first stage of the study, we have formulated and prepared by direct compression, tablets with concentrations of 50 mgs and 100 mgs of metoprolol per tablet (table 1).

After the resulting tablets were tested in quality control and assays, in the last part of the study, we have attempted the simulation of the blocking effect with a slower, respectively faster response from the organism, using two model equations: Verlhust and May.

Table 1: The formulas of the 50 mg and 100 mg metoprolol tablets

Substances	Concentrat n mg/ tablet		Function in formulation
Metoprolol	50	100	Active ingredient
Ludipress	110	212	Support granule
Kollidon VA 64	5	8	Binder, disintegrant
Sodium starch glycolate	4	6	Superdisintegrant
Talcum	3.5	5.5	Lubricant
Magnesia stearate	2.5	3.5	Lubricant
TOTAL	175	335	

For the simulation of the slow response, we have used the Verhulst equation:

$$x_{i+1} = a * x_i * (1-x_i) \quad (1)$$

in which a is a parameter, x_i – the concentration in a certain moment in time, x_{i+1} – the concentration in a later moment. The $a*x_i$ factor is the exponential increase of the concentration, and $(1-x_i)$ is the limitation (constraint) of the possible concentration range.
In order to model a faster response of the biological system, the Robert May equation was used:

$$x_{i+1} = x_i * \exp(k * (1-x_i) \quad (2)$$

The difference form the previous equation is the modification of the variation rule and noting the a parameter as k. [3]

RESULTS AND DISCUSSIONS

Figure 2 shows an overlapping of the variation of concentration in time for the 50 mg tablets. The 3D image clearly shows the variation pattern, with small flutuations from the initial and final values. After the first 5 minutes, the concentration of metoprolol varies from 61.58 % to 65.22 % and after 30 minutes it exceedes 99 %. The variation is nonlinear and can be considered exponential at a first approxiamation. We obtain also very good results for the dissolution rate of metoprolol 100 mg tablets.

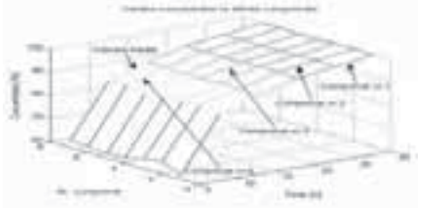

Figure 2: The variation of the concentration for the 50 mg metoprolol tablets

We have attempted to determine the variability of the action of the metoprolol tablets on the human organism, using chaos theory elements.
Initially, in figure 1 we have presented the variation pattern of the metoprolol plasmatic concentrations after ingesting one tablet, using experimental values, correlated to values cited in literature. Figures 2 shows a good enough aproximation of the dissolution time for 50 mg tablets, using an exponential equation, is possible. The maximum concentration value is called *nominal response*. A similar pattern acts during the elimination of the drug, but with different parameters. The values presented in literature are between 1.5 and 4 hours for the first stage, and of 3 to 7 hours for the second (considering the half-life only, because total elimination takes a much longer time).

A significant variability can be observed from ingestion and untill the concentration drops to half of the maximum value, between 4 and 10 hours (figure 3).

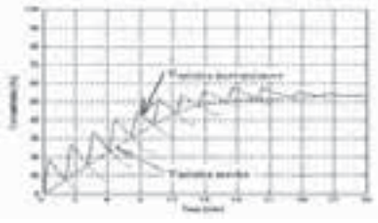

Figure 3: The variation of the metoprolol plasma concentration in time

By eliminating the small variations, the human organism response is better untill reaching a stable state – the red dotted line in figure 8. For organisms with a solwer response, the Verhulst equation (1) can be applied.

The ratio between the tablet administration interval and the minimal value of the recommended interval is noted as a. It can be observed that for small values, corresponding to scarce administration, the values of concentration variation in the organism are between zero and an insufficient maximum (in figure 4, a = 0,05 or a = 0,55). When the value of the ratio is two, the concentration is maintained almost at a constant value. As is exceedes this value, the organism's response begins to oscillate, and if the interval between administrations becomes half of the average recommended interval (the tablets are administered too often), then the concentration may reach double the value obtained for normal administrations, or it may drop to zero. Also, a chaotic response may develop if the administration of the tablets is suddenly ceased. A three-dimensional representation is shown in figure 4.

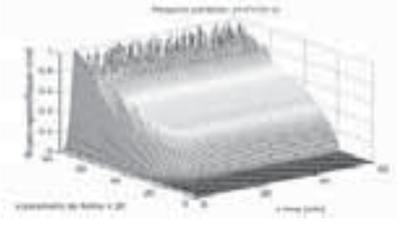

Figure 4: The slow-response of the organism to the variation of the interval between administrations

The study of the influence of the initial concentration, x_i, (fig. 4), and the study of the images presented above show that the system is not significantly dependent on the intervals between administrations.
If the organism's response is a rapid one, the Robert May equation (2) is used.

This type of organism is responding chaotically for smaller deviations than in the previous situation (k = 1.8 compared to a = 2.4).

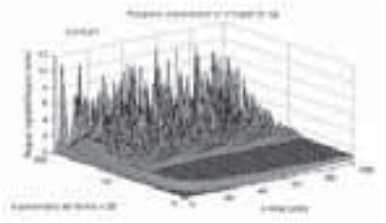

Figure 5: The response of organisms more sensitive to the variation of the intervals between administrations

An almost identical variation could be observed for both types of tablets. In both situations, the dynamic system is stable in some situations: -if the form parameter's value becomes too big, corresponding to a severe shortening of the interval between administrations, the organism responds chaotically, easyly passing from very high concentration values to null values, posing a great risk for the organism; -starting from the quasiperiodical region and to the chaotic region, the analysis shows significant differences of the response to small plasmatic concentration variations (differences of 1 % can be observed).

CONCLUSIONS

In the computer simulation, we have used two equations, one usually used in logistics and another in biology, corresponding to the slower, respectively faster evolutions of the organism's response. Important observations regarding the efficacity of the average concentration variation were made. The curves used for the models have shown three possible stages: when the drug is administered after onger periods – when the value tends towards zero, when the intervals between administrations are normal and when the administration of the tablets is done in a chaotic, aperiodic schedule – when the values are very high or very low.

We consider this study to be of interest because this type of drug is administered orally, for a long period of time, and it is not recommended to suddenly stop its administration.We intend to continue this research in order to determine the relation between the form parameter values and the optimal interval between administrations.

References

1. Hoppensteadt Frank C. Analysis and Simulation of Chaotic Systems, Springer 2001;
2. Addison Paul S *Fractals and Chaos* Napier University, Edinburg, 1997;
3. Picker KM. New insights into the process of tablet formation - Ways to explore soft tableting, Halle, Germany: Martin-Luther-University Halle-Wittenberg, 2002.

PO064

Pharmacokinetic evaluation of different amoxicillin/clavulanate therapeutic systems

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INTRODUCTION

Amoxicillin/clavulanate is considered as a broad-spectrum antibiotic for the treatment of a wide range of bacterial infections and is a widely prescribed combination of beta-lactam antibiotic and beta-lactamase inhibitor available in different conventional dosage forms for peroral delivery and. Amoxicillin is mainly absorbed from the upper small intestine (1) and its bioavailability can be improved by different innovative approaches that enable the drug to retain at the absorption site. Various approaches have been pursued to increase the retention of an oral dosage form in the stomach (2). New therapeutic systems were developed to achieve a prolonged time over MIC of amoxicillin in relation to a regular immediate release amoxicillin/clavulanate formulation.

The objective of the study was to compare pharmacokinetic parameters of several modified amoxicillin/ clavulanate therapeutic systems 1500/125mg or 1600/125mg comprising controlled release (CR) amoxicillin floating capsule and Amoksiklav® 625mg immediate release tablet (500mg amoxicillin and 125mg clavulanic acid) (Lek Pharmaceuticals d.d., Slovenia) under fed conditions.

MATERIALS AND METHODS

Preparation of therapeutic system – IR tablet and CR floating capsule

Amoxicillin/clavulanate IR tablet:

Amoxicillin/clavulanic acid tablet (Amoksiklav® 625mg) or dispersible tablets (Amoksiklav® HR 625mg) including 500mg of amoxicillin in the form of amoxicillin trihydrate and 125mg of clavulanic acid in the form of potassium clavulanate were taken from a regular production batch.

Amoxicillin floating capsule A and B:

Empty preclosed hard capsules from HPMC, Vcaps size #00, natural transparent V001/V001 (Capsugel, France), were film coated with a suspension of Surelease (Colorcon, UK) : Methocel E6 (Colorcon UK,) 60:40 (sample A) or 70:30 (sample B) in a perforated coating pan to apply the dry coat in amount of 6.5 mg/cm^2.

A mixture of 80 g amoxicillin trihydrate (Sandoz, Spain), 9.5 g Methocel K100LV (Colorcon, UK), 9.5 g Avicel PH102 (FMC, USA) and 1.0 g magnesium stearate (Faci, Italy) was blended and tablets of 180mg weight were prepared (tabletT).

560 mg of the same mixture was filled into each coated capsule and the tablet T was added on top of the mixture and the capsule was closed. Each capsule contained 500 mg amoxicillin in the form of amoxicillin trihydrate.

Amoxicillin floating capsule D, E and F:

Empty preclosed HPMC capsules, Vcaps #00, natural transparent V001/V001 (Capsugel, France), were film coated with aqueous dispersion of Surelease (Colorcon, UK) : Klucel EF (Hercules, USA) 50:50 (sample V031A) or ethanolic dispersion of Ethocel N7(Dow Chemical, USA) : Klucel EF (Hercules, USA) 40:60 (sample V036A) in a perforated coating pan to apply the dry coat in amount of 5 mg/cm^2.

A mixture of 32.1 g amoxicillin trihydrate (Sandoz, Spain), 2.4 g Methocel K100LV (granulate 016X) or 2.4 g Methocel K4MP (granulate 017X) (Colorcon, UK), 2.1 g Avicel PH102 (FMC, USA) and 0.4 g magnesium stearate (Faci, Italy) was blended and 180 mg tablets were compressed (tablet T1). 560 mg of the mixture 016X or 017X was filled into each coated capsule and the tablet T1 was added on top of the mixture and the capsule was closed (Table 1).

Each capsule contained 550 mg amoxicillin in the form of amoxicillin trihydrate.

Table 1: Composition of floating capsules D, E, F

	Granulate	Tablet	Capsule
Floating capsule D	016X	T1	V031A
Floating capsule E	017X	T1	V036A
Floating capsule F	016X	T1	V036A

Pharmacokinetic study

Twelve healthy male volunteers, aged 18–45, participated in the studies. The studies were conducted in a randomized, single dose, three period, three formulations, six-sequence crossover design under fed conditions.

The medications used in the first study were:

Therapeutic system A included one IR Amoksiklav® 625mg tablet and two float. caps. A.

Therapeutic system B included one IR Amoksiklav® 625mg tablet and two float. caps. B

Formulation C: One IR Amoksiklav® 625mg tablet.

The medications used in the second study were:

Therapeutic system D included one Amoksiklav® HR 625mg dispersible tablet and two float. caps. D.

Therapeutic system E included one Amoksiklav® HR 625mg dispersible tablet and two float. caps. E.

Therapeutic system F included one Amoksiklav® HR 625mg dispersible tablet and two float. caps. F.

After an overnight fast and 30 minutes before their scheduled dosing times, subjects were received a

standard breakfast. Each volunteer was given an oral dose of the assigned formulation, with 240 mL of water, according to the randomization list. Amoksiklav® HR 625mg dispersible tablets were administered after dispersing in 30mL of water.

Blood sampling schedule: just before drug intake and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14 and 24 hours after drug administration (22 samples per subject per period). Amoxicillin and clavulanic acid in plasma were analysed by HPLC.

The following pharmacokinetic parameters were calculated: AUCt, AUCi, Cmax, tmax, Kel, HL and (AUCt/AUCi)x100, t>MIC 2 and 4 μg/mL.

RESULTS AND DISCUSSION

Mean plasma amoxicillin concentrations of therapeutic systems A, B and C are presented in a figure 1. It is evident that both new therapeutic systems (A and B) have prolonged absorption in comparison with a standard Amoksiklav® 625mg tablet (formulation C).

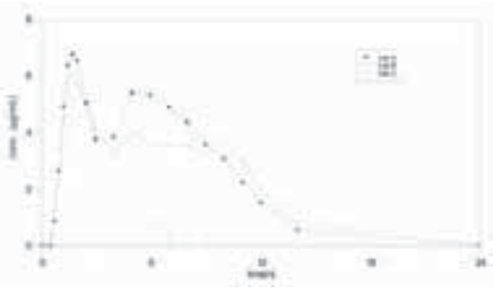

Figure 1: Mean plasma amoxicillin concentrations (formulations A, B and C)

It was expected that in ideal conditions amoxicillin AUCI of therapeutic systems A and B would be three times of AUCI of a standard Amoksiklav tablet. This ratio is almost 3 for a therapeutic system A (the ratio is 2.9), for a therapeutic system B more drug is lost (the ratio is 2.7).

Mean plasma amoxicillin concentrations for therapeutic systems D, E and F (Fig. 2) and other PK parameters (Table 2) prove that all therapeutic systems have prolonged absorption.

Clavulanic acid is in the IR form in all systems and PK parameters are similar for all formulations.

The improvement of the therapeutic efficacy of beta-lactam antibiotics is correlated to the prolonged time above MIC what was also achieved with all therapeutic systems tested (Table 3). All three therapeutic systems meet criteria for time above MIC for twice daily application (3.6 h > MIC4 and 4.8 h > MIC2). PK profiles are improved in comparison to therapeutic systems A and B, while drug concentrations between two peaks (one from IR and another from CR formulation) are higher, Regarding time above MIC, therapeutic systems D, E and F are similar to A and B. When a dispersible tablet for an immediate release part of a therapeutic system is used, the number of formulations that should be swallowed whole, turns from 3 to 2. The lowest tmax and the highest Cmax of the CR part of the drug exhibited therapeutic system F and drug concentrations between two peaks were the highest for the system F among all systems.

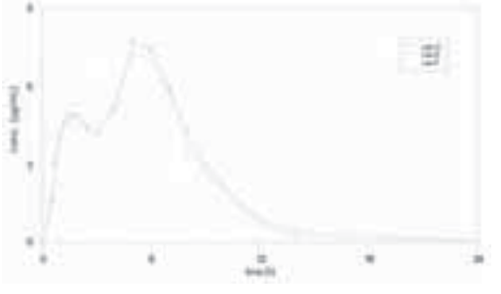

Figure 2: Mean plasma amoxicillin concentrations (formulations D, E and F)

*Table 2: Geometric (*arithmetic for tmax) means of amoxicillin pharmacokinetic parameters*

	Cmax	AUCt	AUCi	tmax*
Ther.system D	9.059	50.473	51.308	5.479
Ther.system E	7.893	50.582	51.516	6.083
Ther.system F	9.928	49.841	50.505	5.333

Table 3: Time of amoxicillin plasma concentrations above MIC.

Therapeutic system	D	E	F
Time above MIC 2 (h)	9.07	9.47	8.45
Time above MIC 4 (h)	6.08	5.84	5.81

CONCLUSIONS

The pharmacokinetic studies proved enhanced pharmacokinetic parameters of a modified amoxicillin/clavulanate therapeutic system comprising IR dispersible tablet and CR floating capsule. A prolonged time over MIC of amoxicillin in relation to amoxicillin/clavulanate immediate release formulations was confirmed.

References

1. W.H. Barr et all, Differential absorption of amoxicillin from the human small and large intestine. Clinical Pharmacol Ther. 56: 279-285 (1994).
2. B.N. Singh, K.H. Kim, Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J. Controll. Release 63: 235-259 (2000).

PO065

Population pharmacokinetic model of topiramate in patients with epilepsy

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INTRODUCTION

Topiramate belongs to the second generation of antiepileptic drugs and has been approved for the treatment of epilepsy (1).

After oral administration absorption of topiramate is rapid with bioavailability ranging from 81 to 95% (2). For doses of topiramate from 100 to 1200 mg the mean apparent volume of distribution is in the range of 0.6 to 1 L/kg (2). At steady state the renal clearance of topiramate was 1.02 L/h (2) and its elimination half- life varies from 20 to 30 hours (1). In patients with epilepsy, treatment with topiramate is commonly associated with CNS side effects. The incidence and severity of many adverse events may be reduced through the slow titration to effective and well tolerated doses (1). Individual factors such as age, renal function as well as concurrent use of other medications can contribute to the pharmacokinetic variability of topiramate (3). In the presence of enzyme-inducing drugs plasma concentrations of topiramate can be reduced by approximately 50% (1). Therefore, monitoring topiramate may be considered at steady state after initiation of treatment to provide an individual reference concentration, at therapeutic failure and in conjunction with drug-drug interactions (3).

The aim of the present study was to develop a population pharmacokinetic (PK) model to evaluate the influence of various factors on pharmacokinetics of topiramate.

PATIENTS

The study population included 26 patients with epilepsy most commonly present in a form of partial or general tonic-clonic seizures or juvenile myoclonic or benign childhood epilepsy. Patients of both genders (male/female=11/15) and various age (adults/children = 17/9) were included. They were on anticonvulsive monotherapy with topiramate (n=15) or polytherapy with topiramate and carbamazepine (n=5) or topiramate and valproate (n=6) for at least two weeks prior to inclusion in the study. The study was approved by the ethic committee of the Clinical center, Kragujevac, Serbia.

BLOOD SAMPLING AND ASSAY

Two blood samples were drawn from each patient in the steady-state. The first blood sample was taken immediately before drug application (trough concentration) and the second was taken around 2 h after the topiramate dosing (approximating peak concentration). Topiramate concentration in plasma was determined by HPLC with fluorescence detection (4).

DATA ANALYSIS

PK analysis was performed by a population PK modeling approach using NONMEM (Version V, GloboMax LLC, Ellicott City, MD, USA) and Visual-NM (Version V, R.D.P.P., Montpellier, France), a Windows based interface to NONMEM. The structural model used was a one-compartment PK model with first-order absorption and elimination. Apparent clearance (CL/F) and volume of distribution (V/F) were estimated, while the absorption rate constant was fixed at 2 h^{-1}, according to literature data (5). Effects of continuous covariates: patient age and weight (WT), body surface area (BSA), daily topiramate dose (DTD), serum creatinine clearance (CL_{Cr}), and liver transaminase (AST, ALT) levels were investigated. Among categorical covariates consid-

ered for inclusion were: patient's gender and co-treatment with carbamazepine (CBZ), valproic acid (VPA) and benzodiazepines (BDZ). Effect of each covariate was tested against the base model. Alternative models were compared by the likelihood ratio test ($\alpha = 0.05$). Significant covariates were rank-ordered and introduced into the full model. The final model was determined by backward elimination of covariates one by one from the full model to see if they should remain in the model using the likelihood ratio test. Additional criterion for retention of a covariate in the model was reduction in unexplained interindividual variability.

The model adequacy was evaluated by standard diagnostic plots, convergence of minimization, number of significant digits more than 3, successful covariance step and gradients in the final iteration in the range between 10^{-3} and 10^{2}.

RESULTS AND DISCUSSION

In the present study the influence of various covariates on topiramate pharmacokinetics was systematically investigated by a population approach. Due to relatively small number of patients included in the study and sparse sampling with only two plasma samples per subject, estimation of all the parameters of the PK model was not possible and the absorption rate constant was fixed to the literature value. Nevertheless, CL/F and V/F could be estimated with reasonable precision. Interindividual variability of CL/F and V/F was modeled using exponential model, while residual, intraindividual variability of topiramate concentration was most adequately described by the additive model. With the base model mean population CL/F (95% CI) was estimated at 1.46 L/h (1.19 – 1.73 L/h) with an interindividual CV of 48.1% (30.9 – 60.5%), and V/F was estimated at 38.8 L (30.7 – 46.9 L) with an interindividual CV of 53.0% (35.9 – 65.8%). Residual variability was 0.11 mg/L (0– 0.17 mg/L). Topiramate CL/F was found to increase with patient age, additionally co-treatment with CBZ increased CL/F by 61% (17 – 105%) (Figure 1).
The final model is described by the following equations:

$$CL/F\,(L/h) = 1.45 \cdot 1.61^{CBZ} \cdot \left(\frac{Age}{30}\right)^{0.36}$$

$$V/F\,(L) = 42.5 \cdot \left(\frac{WT}{70}\right)$$

where CBZ is 1 in patients co-treated with carbamazepine.
In the final model the interindividual CV of CL/F and V/F was 39.8% (24.8 – 50.4%) and 43.9% (32.5-53.0%), respectively, while the residual variability was 0.12 mg/L (0.00 - 0.17 mg/L).

Mean CL/F of topiramate for a typical patient (30 years, 70 kg) on monotherapy with topiramate 200 mg/day was estimated at 1.45 L/h. This is comparable with the findings of previous studies (5), where oral plasma clearance of topiramate was reported to be approximately 1.2 to 1.8 L/h. Topiramate CL/F per patient WT is reported to decrease with age and is approximately 50% higher in children compared to adults (6). However, under the conditions of our study, where pooled population of children and adults were analyzed, WT was co-linear with age.
V/F of topiramate was estimated at 42.5 L. This is in accordance with the results of the previous studies where the mean apparent volume of distribution for doses of topiramate from 100 to 1200 mg was in the range of 0.6 to 1 L/kg (2).

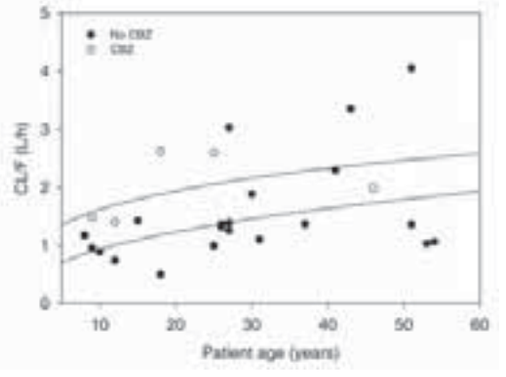

Figure 1: Relationship between individual estimates of topiramate apparent clearance, patient's age and comedication with carbamazepine (CBZ).

Developed population PK model can be used for Bayesian estimation of topiramate PK parameters in individual patients based on sparse therapeutic drug monitoring measurements and consequent individualization of dosing regimen to achieve the target concentration.

References

1. Stefan H, Feuerstein TJ. Novel anticonvulsant drugs. Pharmacol Ther, 2007; 113(1): 165-183.
2. Sachdeo RC. Topiramate-Clinical profile in epilepsy. Clin Pharmacokinet, 1998; 34 (5): 335-346.
3. Johannessen SI, Tomson T. Pharmacokienic variability of newer antiepileptic drugs – When is monitoring needed? Clin Pharmacokinet, 2006; 45 (11): 1061-1075.
4. Bahrami G, Mirzaeei Sh, Kiani A.. Sensitive analytical method for Topiramate in human serum by HPLC with pre-column fluorescent derivatization and its application in human pharmacokinetic studies. J Chromatogr B, 2004; 813(1-2): 175-180.
5. Prescribing information for Topamax Tablets and Sprinkle Capsules. (http://www.topamax360.com/omn360/assets/topamax/pdf/topamax_pi.pdf.
6. Perucca E. Pharmacokinetic variability of new antiepileptic drugs at different ages. Ther Drug Monit, 2005; 27(6): 714-717.

PO066

Efficacy of microparticulated BDS dosage forms in the treatment of experimentally induced colitis in rats

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INTRODUCTION

The most prominent disorders of the large intestine are idiopathic inflammatory bowel diseases, such as ulcerative colitis, a mucosal inflammatory condition confined to the colon and rectum, and Crohn s disease, a transmural inflammation of gastrointestinal mucosa that may occur in any part of the intestine, but mainly occurs in the colon. Development and formulation of new dosage form based on microparticulate systems combining enteric and controlled release properties is a rational approach for efficient treatment of I BD. Introduction of bio/mucoadhesive properties to such dosage form should prolonged its residence time on the inflammation site. This rational could be achieved by formulation of colloidal drug carriers based on chitosan and alginate. Their ability to act as substrate for the bacterial inhabitants of the colon together with their properties, such as swelling, film forming, bio/mucoadhesion and biocompatibility invites their use as colon carriers.

We have designed a new microparticulate system loaded with budesonide (BDS), consisting of a chitosan-Ca-alginate polymer matrix, with sustained-release properties, coated with an acrylic polymer (Eudragit S 100) with pH-dependent swelling and dissolution properties [1].

The aim of this work was to determine the efficacy of the microparticulate systems containing BDS in the treatment of experimentally induced colitis in rats. The effect of eudragit coated microparticles containing BDS was compared with those obtained after administration of uncoated chitosan-Ca-alginate microparticles with BDS and suspension of BDS alone.

EXPERIMENTAL METHODS

The experiments were approved by the ethical committee of the Institute of Biology, Faculty of Natural Sciences and Mathematics

Induction of colonic inflammation

To induce the model of chronic inflammation in rat colon, we followed the method described by Morris et al. [2].

Experimental design and dosing

At 24 h after the induction of colonic inflammation, each treatment group (*n=5* rats) was administered one of the following formulations by oral gavage, once a day for 5 days: BDS suspension (BDSs); BDS loaded chitosan–Ca–alginate microparticles (MPB) and BDS loaded Eudragit S coated chitosan–Ca–alginate microparticles (E-MPB). Rats were administered a dose of 167 μg/ kg/ day BDS, i.e. quantity of microparticles containing BDS equivalent to this dose. In all cases, drug or microparticles were suspended in 1 mL purified water. Rats were sacrificed 6th day after the intracolonic administration of TNBS.

The effects of administered samples were evaluated by determination of of colon/bodyweight ratio [3], assessment of macroscopic ulceration and histological evaluation [4], and clinical activity score system [5].

Statistical analysis

The results are expressed as mean values ± SD. For analysis of statistical significance a one-way analysis of variance (*ANOVA*) was applied. In all cases $p < .05$ was considered to be significant.

RESULTS AND DISCUSION

Index of colonic tissue oedema (colon/body weight ratio) is presented on Fig.1.

Clinical activity score points were 2.2 ± 0.12 for control (non treated, TNBS) group, 2.2 ± 0.23 for BDSs, 2.07 ± 0.26 for MPB and 1.63 ± 0.49 for E-MPB, respectively (Fig.2).

Histological findings for control (non treated, TNBS) group showed complete destruction of mucosa structure, ulceration and necrosis, while for BDSs group showed necrosis with demarcation signs and formation of granulation tissue.

Histological findings for MPB group showed focal ulceration, signs of regeneration, and part with normal mucosa structure, while for E-MPB group showed formation of large granulation tissue indicating strong regeneration tendency.

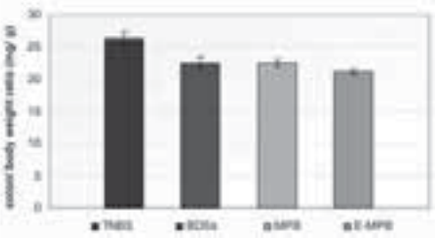

Figure 1: Index of colonic tissue oedema (colon/body weight ratio) of animals with TNBS induced colitis after treatment with BDSs, MPB and E-MPB and for animals which after the induction of colitis with TNBS did not receive any therapy (TNBS), (mean ± SD, n=5)

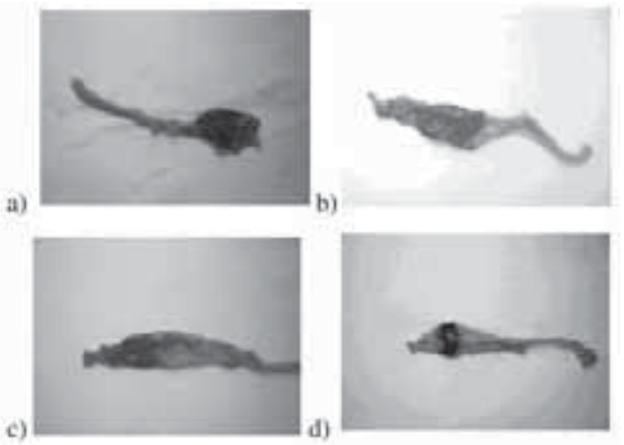

Figure 2: Photographs of the colon of rat a) control (non treated, TNBS) group, clinical activity/ score points 2.20 ± 0.12; b) treated with BDSs; clinical activity/ score points 2.20 ± 0.23; c) treated with MPB; clinical activity/ score points 2.07 ± 0.26; d) treated with E-MPB; clinical activity/ score points 1.63 ± 0.49

Macroscopic ulceration and histological evaluation score points were 6±0 for control (non treated, TNBS) group, 5±0.55 for BDSs, 4.33±0.41 for MPB and 3.67±0.52 for E-MPB, respectively.

Results of statistical analysis of total score points, and also, individual clinical and histological evaluation, showed that incorporation of drug substance in microparticles had significant differences in favour of efficacy of designed dosage form with bio/mucoadhesive and controlled release properties (oneway *ANOVA*, $p < .05$).

CONCLUSION

Polymeric particulate carrier systems are expected to target the inflamed tissue in inflammatory bowel diseases. Enteric coated chitosan-Ca-alginate microparticles compared with uncoated microparticles and drug suspension significantly improved efficacy of budesonide in the healing of induced colitis in rats. Physical properties of evaluated microparticulate system are in favour of drug accumulation in the inflamed tissue, enhancing the effect of administered dose. Moreover, the sustained drug release allows pharmacological effects to be extended due to the prolonged residence time of the carrier system at the targeted inflamed area. The described system may therefore be useful for clinical treatment of human colonic inflammatory bowel disease.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of NATO SfP: 978023.

References

1. Simonoska Crcarevska M., Glavas Dodov M., Goracinova K., Chitosan coated Ca-alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa. Eur J Pharm Biopharm 68(3): 565-578 (2008)
2. Morris G.P., Beck P.L., Herridge M.S., Depew W.T., Szewczuk M.Y., Wallace J.L., Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterol I 96: 795-803 (1989)
3. Yue G., Sun F.F., Dunn C., Yin K., Wong P.Y.-K., The 21-aminosteroid tirilazad mesylate can ameliorate inflammatory bowel disease in rats. J Pharmacol Exp Ther 276: 265–270 (1996)
4. Rodriguez M., Antonez J.A., Taboada C., Seijo B., Torres D., Colon-specific delivery of budesonide from microencapsulated cellulosic cores: evaluation of the efficacy against colonic inflammation in rats. J Pharm Pharmacol 53 (9): 1207–1215 (2001)
5. Lamprecht A., Ubrich N., Yamamoto H., Scheafer U., Takeuchi H., Maincent P., Kawashima Y., Lehr C.M., Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. J Pharmacol Exp Ther 299: 775–781 (2001)

PO067

Formulation and in vitro in vivo evaluation of triclosan coated grafts

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INTRODUCTION

Polypropylene grafts are commonly used in hernia repair and abdominal wall reconstruction because of their lower recurrence rate and simple application (1). However, graft infection may be a significant problem in up to 10 percent of operations. Triclosan is used in cosmetic and medical industry for more than 30 years as an antibacterial agent (2,3). The purpose of this study was to develop and characterize triclosan coated grafts and evaluate the antibacterial activity of grafts on hernia graft infection model.

EXPERIMENTAL METHODS

• Preparation of Formulations

1% chitosan (low, medium and high molecular weight; Fluka, Buchs, Switzerland) were dissolved in 1% acetic acid solution until a clear gel was obtained after overnight stirring. Then, 1% triclosan was dissolved in this gel. Poloxamer gel was prepared according to the cold method (4). Mucoadhesive polymer HPMC (0.2%) and Pluronic F127 (20%) were dissolved with gentle mixing overnight at 4ºC. Grafts were cut into 2x2 cm squares, incubated in gels for 24 hours at room temperature and dried in a vacuum oven.

• In Vitro Release Studies

Release profiles of triclosan from grafts were determined in 100 mL of isotonic PBS (pH 7.4) containing 1% SDS providing sink conditions in a thermostated shaker bath system (Memmert, Schwabach, Germany) at 37°C. At predetermined time intervals, samples were withdrawn from the system and replaced with equal volume of fresh release medium

maintained at the same temperature. The released amount of triclosan was determined by a spectrophotometric assay at 281 nm using a Shimadzu UV–VIS 160A spectrophotometer (r^2=0,999).

• In Vivo Studies

In the light of in vitro release data, optimum formulation with the controlled release profile selected for in vivo studies was high molecular weight chitosan gel containing triclosan. Minimal inhibitory concentration of microorganisms was evaluated against S.Aureus ATCC29213, inhibiting the growth of this bacterium concentrations of $2x10^7$ colony-forming units (CFU)/ml, with triclosan concentrations up to 10^{-15} of the initial concentrations (10 mg/mL). Thirty-two Wistar albino male rats were divided into 4 groups (Table 1). Groups were; naive grafts (Group 1), naive grafts with antibiotic prophylaxis (Group 2), grafts coated with only chitosan (Group 3), grafts coated with triclosan loaded chitosan gel (Group 4). On the eighth day, the animals were sacrificed and the grafts were removed. The isolated suspensions were cultured and grafts were observed for adherence under scanning electron microscope.

Table 1: In vivo study groups

Groups	Drugs	i.p. antibiotic	rat (n)
Control	Blank graft	No	8
Group 2	Blank graft	Teicoplanin	8
Group 3	Chitosan	No	8
Group 4	Chitosan+		
	Triclosan	No	8

• Surface Morphology of Grafts

A scanning electron microscope was used to evaluate surface characteristics of grafts. Grafts were mounted on the metal stubs with conductive silver paint and then sputted with a 150A° thick layer of gold in a Bio-Rad apparatus.

RESULTS AND DISCUSSION

• In Vitro Release Studies

The release of triclosan was up to 11 μg/mL within 24 hours from the grafts and lasted for 7 days from poloxamer gel. For chitosan gels, the release was determined to be 4, 5, 8 μg/mL for medium, low and high molecular weight chitosans, respectively.

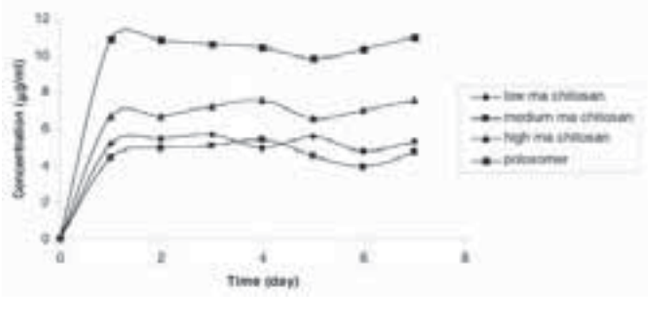

Figure 1: In vitro release profiles of triclosan from the grafts

• In Vivo Studies

When the rats were evaluated for infection, it was observed that there was not any abscess for triclosan loaded chitosan gel grafts. On the contrary, no significant difference was observed when compared to the other groups (Figure 2).

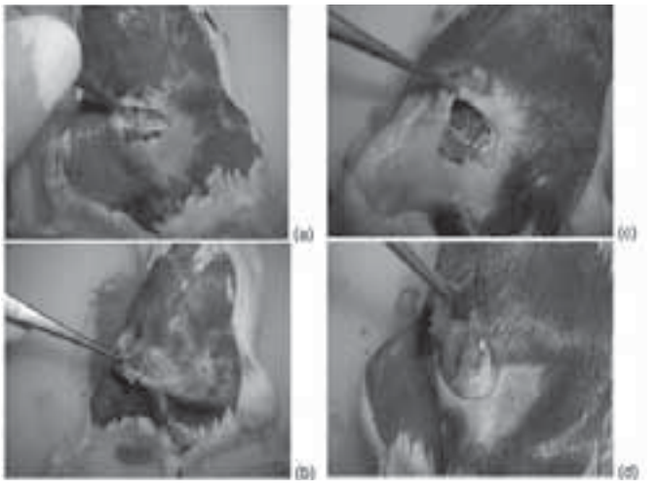

Figure 2: Inflammation evaluation of rats (a: Group 1, b: Group 2, c: Group 3, d: Group 4)

• Surface Morphology of Grafts

Imaging of the grafts by SEM was expected to provide information on morphology. SEM images of the grafts were presented in Figure 3. Examination of SEM photographs of the grafts revealed that triclosan loaded chitosan gel grafts significantly reduced the bacterial adherence compared with Groups 1, 2 and 3.

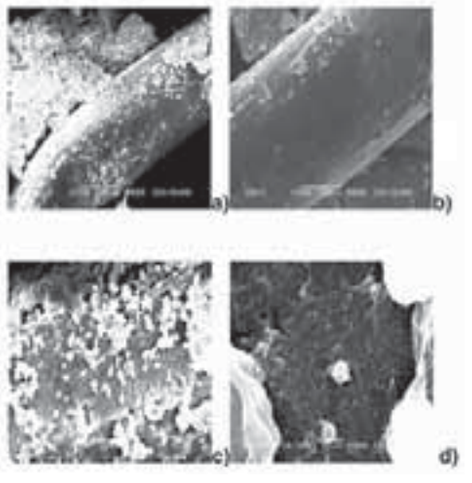

Figure 3: SEM photographs of the grafts (a: Group 1, b: Group 2, c: Group 3, d: Group 4)

CONCLUSION

This study revealed that triclosan coated grafts were effective against the reduction of bacterial adherence to polypropylene grafts and further graft infection.

ACKNOWLEDGEMENT
This project is supported by a grant from the Hacettepe University Research Project.

References

1. Yerdel MA, Ak n EB, Dolalan S, et al.: Effect of single-dose prophylactic ampicillin and sulbactam on wound infection after tension-free inguinal hernia repair with polypropylene mesh. Ann Surg., 233; 26-33 (2001)
2. Jones RD, Jampani HB, Newman JL, et al.: Triclosan:a review of effectiveness and safety in health care settings. Am J Infect Control., 28; 184-196 (2000)
3. Bhargava HN, Leonard PA: Triclosan: applications and safety. AJTC Am J Infect Control., 24; 209-218 (1996)
4. Bilensoy E, Cirpanli, Y., Sen, M., Dogan, L., Calis, S.: Thermosensitive mucoadhesive gel formulation loaded with 5-Fu:cyclodextrin complex for HPV-induced cervical cancer. J Incl Phenom Macrocycl Chem., 57; 363-370 (2007)

PO068

Natural surfactant-based topical vehicles for two model drugs: influence of different lipophilic excipients on in vitro/in vivo skin performance

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INTRODUCTION

Over the past decade, there has been a growing interest into new skin- and environment-friendly (natural) surfactants. In order for a new mixed emulsifier to be accepted as an official pharmaceutical excipient for topical products, a number of critical data are required. These include a comprehensive physicochemical characterization of the variety of different formulations, a study of the impact of those formulations on *in vitro* release/permeation and *in vivo* efficacy for a number of model drugs, as well as an evaluation of the safety profiles of its formulations [cf. 1].

This study focuses on the properties of vehicles based on alkylpolyglucoside natural surfactant-mixed emulsifier, cetearyl glucoside, mixed with cetearyl alcohol. Our previous studies presented a detailed physicochemical and *in vitro/in vivo* characterization of a range of vehicles, as well as active samples containing hydrocortisone or urea as model drugs [2, 3]. In this study, we were interested to investigate further how the alternative use of three lipophilic excipients (Ph. Eur. 6.0), differing in their polarity indexes (medium chain triglycerides – MG, decyl oleate - DO and isopropyl myristate - IPM, resp.), in the alkylpolyglucoside-based vehicles affects *in vitro* permeation profiles of two model drugs: diclofenac sodium (DC) and caffeine (CF), both sparingly soluble in water. Finally, we aimed to evaluate the safety profile of such vehicles *in vitro*, by a cytotoxicity assay, comparing it with *in vivo* data obtained by the methods of skin bioengineering.

EXPERIMENTAL METHODS

• Materials / Preparation of the samples

The alkylpolyglucoside cetearyl glucoside and cetearyl alcohol (Montanov® 68 PHA, Seppic, France) was used in a fixed concentration of 7% (w/w) for the preparation of samples of three model creams (with 17% (w/w) of oil, preserved water up to 100%)), labeled as follows: MG-PL, DO-PL, IPM-PL. Active samples contained dissolved model drugs: 1% (w/w) of DC or 2% (w/w) of CF, alternatively.

• In vitro permeation study through artificial skin constructs (ASCs)

ASCs were cultivated according to the previously described procedure [cf. 4]. In vitro permeation studies were carried out with modified Franz cells (n=6), according to ref. [2]. HPLC determination of DC and CF was done using a Waters 515 / 717plus / 486 HPLC system (Waters, D-Eschborn).

• In vitro skin irritation test - cytotoxicity assay

A modified version of Mosman´s MTT method was used, as it was previously reported in details [4, 5].

• Skin bioengineering evaluation

Three skin parameters were measured in 10 human volunteers before application of test samples and upon a 24h-occlusion: erythema index (EI), skin hydration (SH) and transepidermal water loss (TEWL). Effects of placebo samples (MG-PL, DO-PL, IPM-PL) were compared mutually and related to non-treated control under and without occlusion (NCO, i.e. NCWO) by the Wilcoxon matched paired signed rank test ($p<0.05$).

RESULTS AND DISCUSSION

Despite the different rheological characteristics (data not shown), the samples have not varied significantly in their permeation profiles for both drugs in the first 8 h of the experiment (Fig. 1), except the sample containing DO as oil phase and CF as model drug. Interestingly, both DO-containing samples have showed the lowest steady-state fluxes (2.40 ± 1.26 g/cm^{2}s for DO-DC i.e. 5.50 ± 0.95 g/cm^{2}s for DO-CF), as well as the permeation coefficients. However, in the later phases of ex-

periment significant differences have appeared in permeation profiles for both model drugs; the highest permeation was seen in samples with MG, whereas other two lipophilic excipients had different effect dependent on incorporated drug (Fig. 1).

Distinguished permeation profiles could be partly attributed to the interaction between the vehicle components and the skin-construct (ASC) membrane.

Concerning the *in vitro* skin irritation test all samples showed a concentration-dependent decrease in ASC cell viability, but keeping it over 50% all the time (Fig. 2).

Namely, no one sample exhibited significant effect on the cell viability, thus supporting obtained *in vivo* results on the mildness of alkylpolyglucoside surfactants [2].

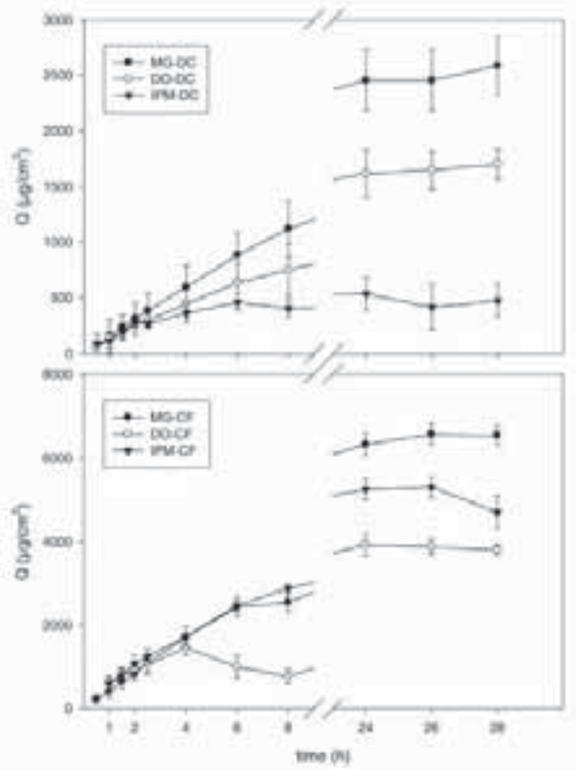

Figure 1: The influence of different oils on ASC-permeation profiles of DC (up) and CF (down)

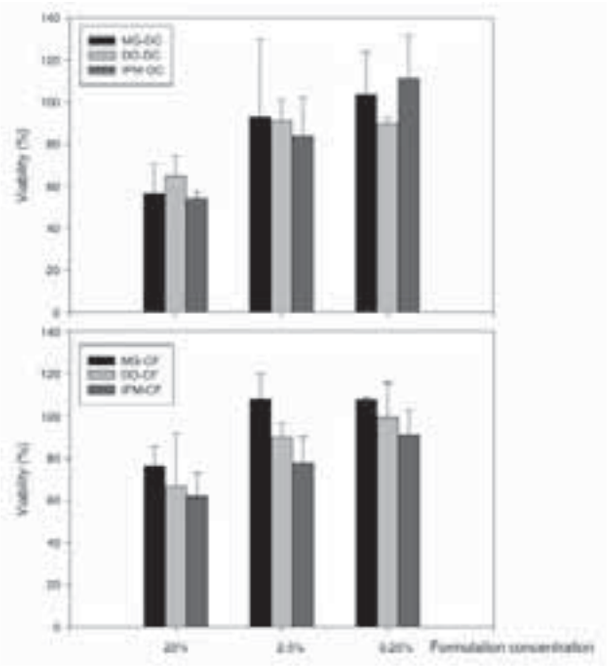

Figure 2: Concentration-viability histograms for DC (up) and CF (down) loaded samples

Throughout the *in vivo* study (data not presented), no adverse reactions related to test samples were recorded.

All test samples tended to decrease EI and TEWL values, although insignificantly; on the other side, skin hydration increased in all, but most prominently in the DO-containing samples. Thus, comparison of these *in vivo* results with our *in vitro* cytotoxicity assay reveals a correlation between the two.

CONCLUSION

Overall, obtained permeation and irritancy test results fully support the suitability of cetearyl glucoside and cetearyl alcohol mixed emulsifier as a prospective pharmaceutical excipient for topical use.

References

1. J. Hadgraft. Eur. J. Pharm. Biopharm. **58:** 291-299 (2004).
2. S. Savic et al. Eur. J. Pharm. Sci. **30:**441-450 (2007).
3. Savić S, Tamburić S, Jančić B, Milić J, Vuleta G, In: T. Tadros, Colloid and Interface Science Vol. 4 (1. ed.), Wiley, February 2008., pp. 259-274.
4. C. Hoffmann, C.C. Müller-Goymann. Pharmazie **60(5):** 350-353 (2005).
5. T. Mosmann. J. Immunol. Methods **65**: 55-63 (1983).

PO069

Effect of DMSO, acetonitrile, ethanol and methanol on cell viability

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INTRODUCTION

In the drug discovery and development process compounds are often poorly soluble in water, what hampered their pharmacological and toxicological characterization. This problem is solved by a routine dissolution of compounds in water-miscible organic solvents, e.g. dimethyl sulfoxide (DMSO). However, organic solvents influence in a concentration dependent manner cell physiology ranging from changed gene expression [1] and enzyme activity [2] to deleterious impact on cell viability [3]. The aim of the present study was to assess the impact of four frequently used organic solvents, e.g. DMSO, acetonitrile, ethanol and methanol, on cell viability after 24h exposure period.

EXPERIMENTAL METHODS

Cell culture

ChoK1 cell line was cultured in DMEM high glucose media (Sigma, 90%) supplemented with fetal bovine serum (Invitrogen, 10%) and adjusted to contain 2mM glutamax (Invitrogen) and 0.1mM non essential amino acids (Invitrogen). JurkatE6.1 cell line was cultured in RPMI media (Sigma, 90 %) supplemented with fetal bovine serum (Invitrogen, 10%) and adjusted to contain 2mM glutamax (Invitrogen), 1mM sodium pyruvate (Invitrogen) and 10mM HEPES (Invitrogen). Cells were cultured in exponential growth phase at 37°C, 5% CO_2 and 95% humidity.

Table 1: Solvent TC50 values. Values in parenthesis represent 95% confidence interval. TC50, concentration of solvent, which reduces cell viability for 50%.

		JurkatE6.1 cell line		
		ATP assay	Resazurin assay	AK release assay
TC50 (% v/v)	DMSO	1.1 (1.0-1.2)	1.1 (1.0-1.2)	9.3 (7.1-12.1)
	Ethanol	0.6 (0.5-0.7)	0.6 (0.5-0.7)	5.0 (4.3-5.8)
	Methanol	0.9 (0.7-1.0)	1.1 (1.0-1.2)	11.6 (9.9-13.5)
	Acetonitril	5.3 (4.6-6.0)	5.4 (4.8-6.0)	12.0 (ND[a])

		ChoK1 cell line		
		ATP assay	Resazurin assay	AK release assay
TC50 (% v/v)	DMSO	2.3 (1.7-3.1)	3.6 (2.9-4.5)	3.9 (2.8-5.5)
	Ethanol	2.7 (2.6-2.8)	2.7 (2.3-3.2)	6.0 (4.7-7.6)
	Methanol	2.3 (1.9-2.7)	2.5 (2.1-2.9)	4.8 (2.8-8.2)
	Acetonitril	4.6 (3.8-5.5)	5.2 (4.0-6.8)	8.8 (8.0-9.7)

[a], too wide 95% confidence interval

Cytotoxicity assays

Solvents were added on a white 96 well plate prior to the seeding of JurkatE6.1 cells or 24h after the seeding of ChoK1 cells. JurkatE6.1 cells were seeded at 25000cells/100 µl/well, while ChoK1 were seeded at 12500cells/100 µl/well. After solvents addition plates were incubated for 24h. At the end of incubation period three cytotoxicity endpoints were determined in each well, namely adenylate kinase (AK) release (Lonza), reduction of resazurin into resorufin (Promega) and intracellular determination of ATP (Promega). Briefly, 20 µl of supernatant was transferred into a new white 96 well plate followed by the addition of AK release reagent (100 µl/well), equilibration at room temperature for 5min and luminescence recording. To the each well of the original plate 20 µl of resazurin reagent was then added followed by 2h incubation and fluorescence reading. Finally, the intracellular amount of ATP was determined by addition of ATP reagent (100 µl/well) followed by equilibration at room temperature for 10min and luminescence recording. Cell viability was calculated relative to the no solvent group.

RESULTS

Fig. 1 shows a concentration dependent cytotoxic effect of tested solvents. The rank orders of cytotoxicity acquired by ATP, resazurin and AK assay were obtained on the basis of solvent concentration which reduced cell viability for 50% (TC50) (Table 1). Although TC50 values varied among assays, a common magnitude order of the cytotoxic effect in the JurkatE6.1 cell line was ethanol>methanol=DMSO>acetonitrile and in the ChoK1 cell line ethanol=methanol=DMSO>acetonitrile (Table 1). The highest TC50 values were obtained by the AK assay, which reflects late stage of cytotoxic membrane rupture event. As judged by the AK and resazurin assay tested solvents did not significantly reduce cell viability ($p>0.05$, ANOVA) in ChoK1 cell line at concentration up to 1% v/v. But in a more sensitive JurkatE6.1 cell line DMSO and methanol caused a significant decrease of cell viability ($p<0.05$, ANOVA) already at concentration of 1% v/v. On the other hand, a more sensitive ATP assay revealed that ethanol, methanol, acetonitrile and DMSO significantly decreased cell viability ($p<0.05$, ANOVA) in the ChoK1 cell line at concentrations above 0.25% v/v, 2 % v/v, 1% v/v and 0.5% v/v, respectively, and in the JurkatE6.1 cell line at concentrations above 0.5% v/v, 0.5% v/v, 2% v/v and 0.5% v/v, respectively.

CONCLUSION

Sensitive ATP assay revealed that cell viability was significantly decreased by all tested solvents at concentrations close to or above 1% v/v. Among the evaluated solvents acetonitrile was the least cytotoxic as judged by the highest TC50 values. Therefore it would be worth to consider the usage of acetonitrile as organic solvent of choice for *in vitro* drug characterization studies. However, the most often used water-miscible organic solvents for *in vitro* drug characterization studies is DMSO at 1% final concentration. By using DMSO at such concentration cell physiology is affected, what might hamper the correct interpretation of the results.

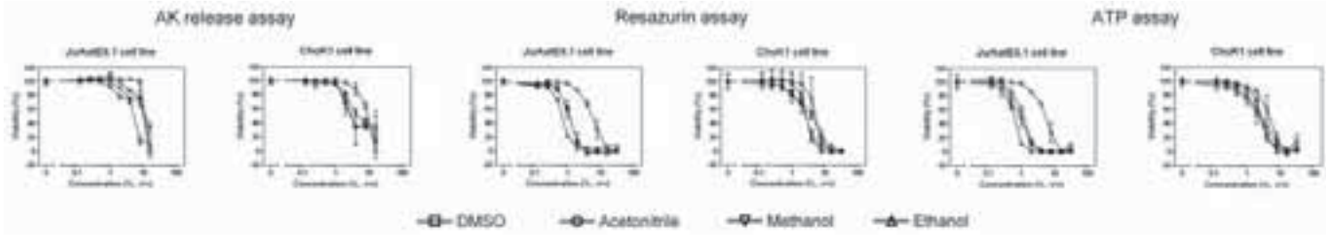

Figure 1: The effect of tested solvents on cell viability after 24h exposure period. Data are shown as mean ± SEM (n=4).

References

1. Zhang W, Needham DL, Coffin M, Rooker A, Hurban P, Tanzer MM, Shuster JR. Microarray analyses of the metabolic responses of Saccharomyces cerevisiae to organic solvent dimethyl sulfoxide. J Ind Microbiol Biotechnol 30: 57-69 (2003).
2. Easterbrook J, Lu C, Sakai Y, Li AP. Effects of organic solvents on the activities of cytochrome P450 isoforms, UDP-dependent glucuronyl transferase, and phenol sulfotransferase in human hepatocytes. Drug Metab Dispos 29: 141 - 144 (2001).
3. Miret S, De Groene EM, Klaffke W. Comparison of in vitro assays of cellular toxicity in the human hepatic cell line HepG2. J Biomol Screen 11: 184 - 193 (2006).

PO070

Red blood cells as bioreactors for ethanol processing

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INTRODUCTION

For the first time, enzyme-loaded red blood cells (RBCs) were proposed for enzyme therapy of certain diseases. It is possible that the consequences of these diseases could be reduced if replacement enzymes could be introduced into the patients to catalyze the missing reaction. However, the injection of free enzyme can be ineffective because of the possibility of either immune response or because the enzyme will be rapidly cleared from bloodstream. These difficulties led to the idea of encapsulating enzymes in some kind of semipermeable envelope that would retain enzyme while allowing substrates to enter. As such carrier, RBCs can be used. They can be extraordinary vehicle for the enzymatic degradation of elevated concentration of substances in the blood circulation in other words – bioreactors. Bioreactors are RBCs, in which enzyme or enzymes, which are not always present initially, are loaded. Loaded enzymes become a part of RBC metabolic pathways. In our work, human RBCs with coencapsulated enzymes (alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AdDH)) were created as bioreactors (alcocytes) for the removal of elevated blood ethanol and acetaldehyde concentration (Fig.1). Since the main application of alcocytes is toxic concentration decrease, so an important value of their function is ethanol processing rate. The rate of ethanol disappearance from blood is 5.4-10.8 mM/h [1]. For effective alcocytes function, the ethanol oxidation rate should be comparable with liver functioning rate. It follows from the results of Lizano C. et al. [2] that RBCs-bioreactors processing ethanol in vitro with the rate 1.7 mM/h (Ht=100%) correspond to a rate of 0.11 mM/h in blood after their transfusion.

The objective of this work was to increase the ethanol oxidation rate in vitro by alcocytes.

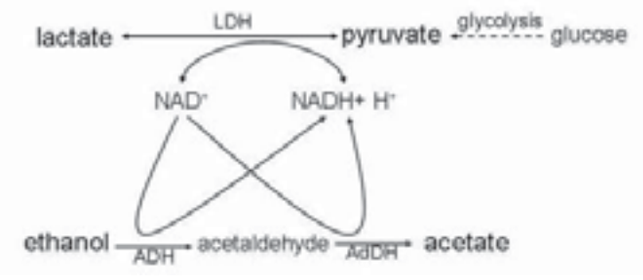

Figure 1: The scheme of bioreactor function. Alcohol dehydrogenase (ADH) catalyzes ethanol oxidation to acetaldehyde, and further aldehyde dehydrogenase (AdDH) metabolizes acetaldehyde to acetate, both reactions pass with NAD^+ reduction. These enzymes employ glycolysis for their function, to oxidize generated NADH to NAD^+ by RBCs resident lactate dehydrogenase (LDH).

METHODS

RBCs-bioreactors were obtained with the method of two-steps dialysis. This method based on the swelling of the cells accompanied by an increase in the permeability of the membrane of the RBCs when it is exposed to a hypotonic solution. The encapsulation of the enzymes is

favoured because pores appear in the membrane when RBCs are under reduced osmotic pressure conditions. Dialysis was performed on a developed laboratory apparatus based on a standard dialyzer. For recovery of NAD loss, which occurs during dialysis, NAD was added to hypotonic solution.

RESULTS AND DISCUSSION

A laboratory equipment was developed for loaded RBCs production. This apparatus allows to obtain loaded RBCs in quantities sufficient for clinical use. By means of this equipment, the alcocytes for ethanol processing with encapsulation yield of 70% were received. For evaluation of RBCs-bioreactors function, experiments on ethanol decrease in vitro were performed. It was shown that alcocytes process ethanol with the rate of 32 mM/h (Ht=100%). Taking into account dilution at alcocytes transfusion, the rate of their functioning will be 2 mM/h, which is a lot higher than the rate obtained by other authors and has the same order of magnitude that the rate of ethanol metabolism by liver. Such rate increase is provided by concentration recovery of NAD^+ in RBCs, which occurs during dialysis, and optimal ratio of ADH and AdDH in alcocytes.

References

1. Kater R.M.H, Carulli N., Iber F.L. Differences in the rate of ethanol metabolism in recently drinking alcoholic and nondrinking subjects. Am J Clin Nutr 22(12): 1608-1617 (1969).
2. Lizano C., Sanz S., Luque J., Pinilla M. In vitro study of alcohol dehydrogenase and acetaldehyde dehydrogenase encapsulated into human erythrocytes by an electroporation procedure. Biochim Biophys Acta 1425: 328-336 (1998).

PO071

Modelling dissolution of weakly basic drugs in physiological environment

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INTRODUCTION

Weakly basic drugs are characterized by good solubility in acidic media and low solubility in alkaline media. Therefore in physiological conditions it is expected that the main dissolution of the drug will take place in stomach environment, where the pH value is low. However, the dissolution of weakly basic drugs, whose solubility changes substantially in the range of physiological pH values, can be influenced by the varying pH values in the stomach environment after liquid or food ingestion. Therefore we have developed a mathematical model for predicting drug dissolution in stomach environment which takes into account variable pH values in fasted or fed state as well as the stomach emptying into the intestine. In addition, the model accounts for drug dosage form parameters such as particle size distribution and disintegration properties. In this presentation we describe the mathematical model and analyze the effect of different physiological and formulation parameters on drug dissolution for a model drug compound.

MODEL

Physiological parameters

In typical fasted state studies the drug is ingested with about 200 ml of water. The pH in the stomach after the ingestion raises up to 4.5. It takes about 30 minutes for pH in the stomach to reach the value of 2.0 and about 60 minutes to return to the equilibrium value of 1.5. Simultaneously the contents of the stomach is emptied into the intestine with a half time of approximately 15 minutes [1,2].

In fed state studies the volume of stomach contents after the food ingestion is typicaly about 1000 ml. The pH in the stomach immediately after the ingestion can be as high as 7.0. It takes about 45 minutes for pH to decrease to the value of 3.0 and about 90 minutes to reach the value of 2.0. The contents of the stomach is emptied into the intestine with a half time of about 60 minutes [3].

Dissolution of drug and formulation parameters

Dissolution of drug particles is modeled using Fick s first law of diffusion. For spherical particles the decraese of the particle radius while dissolving is described by differential equation

$$dr = -\frac{D}{\rho}\frac{(c_s - c_b)}{h}\left(1+\frac{h}{r}\right)dt.$$

Here D is the diffusion constant, ρ the density of solid particle, c_s the drug solubility, c_b the drug concentration in dissolution medium, h the diffusion layer thickness and r the particle radius. The solubility c_s depends on the pH of the environment and therefore changes with time as the pH in the stomach varies after liquid or food ingestion.

In the presented case we assume that the drug is formulated as an immediate release tablet. The particle size distribution of drug substance and the disintegration rate of the tablet can be taken into account. As a model compound we choose a weakly basic drug with the pKa = 3.5 and intrinsic solubility S_0=0.016 mg/ml. The solubility of the compound is then given by the solubility equation

$$S = S_0(1 + 10^{pKa-pH})$$

and varies considerably in the range of physiological pH values encountered in stomach environment.

RESULTS

Figure 1 shows the dissolution of the drug in the stomach for an immediate release tablet. The size of drug particles (radius) is taken to be 25 microns and the tablet is assumed to disintegrate immediately. We model the dissolution for the fed state of stomach. We consider two cases with a constant pH in stomach environment (pH 2.0 and pH 5.0) and the case of a time varying pH, which describes the *in-vivo* situation more realistically.

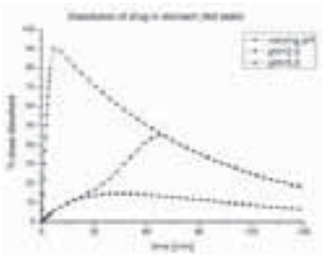

Figure 1: The dissolution of drug in stomach: a) assuming constant pH values 2.0 and 5.0; b) varying pH. The amount of dissolved drug in stomach is plotted on ordinate axis. The stomach emptying is taken into account.

In the case of low pH value (pH 2.0), where the solubility is high, the drug dissolves very quickly. In the opposite case with pH 5.0, where the solubility is low, only a small amount of drug dissolves in the stomach while the majority is emptied into the intestine undissolved. In the case of varying pH the drug dissolution is much slower than in the case of low pH (2.0) but also considerably faster than in the case of high pH (5.0). This shows that when predicting dissolution of weakly basic drugs in stomach environment, realistic physiological conditions (i.e. time changing pH value) should be considered. Otherwise the dissolution rate and the extent of dissolution can be grossly over- or underestimated. The same conclusion also applies to the case of fasted state and for smaller particle size of drug substance.

Using the above described model we analyzed plasma concentration data obtained from *in-vivo* studies which tested two different formulations of the drug. We first extracted the expected *in-vivo* dissolution profiles from the plasma concentration profiles. Then the dissolution profiles were simulated using the presented mathematical model. The influence of different formulation parameters (particle size distribution, disintegration, effective solubility) on the dissolution profiles was analyzed. We found that the dissolution of the drug is primarily determined by the disintegration properties of the tablet. The difference in the effective solubility of the drug (due to interaction with excipients) and particle size distribution seem not to affect the dissolution considerably. Figure 2 shows the comparison between the *in-vivo* dissolution of the drug and simulated dissolution profiles. Different parameters describing disintegration of the two tested formulations were used in the simulation.

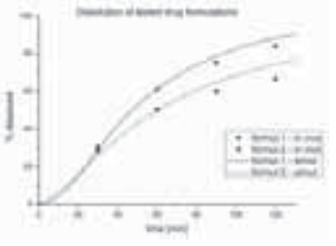

Figure 2: Dissolution of the two tested formulations (fasted state conditions). Presented is the amount of dissolved drug in the intestine, where the absorption takes place. Simulated results are compared with in-vivo data.

CONCLUSION

With the presented mathematical model we demonstrated that realistic physiological conditions should be considered when predicting the dissolution of weakly basic drugs in stomach environment. Otherwise the dissolution can be grossly misinterpreted. On the presented example we demonstrated how modelling can be used as a tool to analyze the influence of different formulation parameters on *in-vivo* drug dissolution.

References

1. S. Souliman et al., Eur. J. Pharm. Sci. 27 (2006), 72-79
2. D. Adkins et al, Br. J. Clin. Pharmac. 39 (1995), 381 -387
3. J. B. Dressman et al., Pharm. Res. 7 (1990), 756-761

PO072

Biopharmaceutical evaluation of clopidogrel salts

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INTRODUCTION

Salt formation is well-known technique to modify and optimize the physical chemical properties of an ionizable compound. Properties like solubility, dissolution, hygroscopicity, physical and chemical stability, impurity profile as well as polymorphism can be changed [1].

Clopidogrel is an anticoagulant drug that inhibits platelet aggregation by selective binding to adenylate cyclase-couplet ADP receptors on the platelet surface. It is widely used for the prevention of atherothrombic events [2].

Clopidogrel free base is not stable due to a labile proton in the chiral center and is susceptible to racemization and hydrolysis of a methyl ester group. Currently, the bisulphate salt form of Clopidogrel is on the market.

Clopidogrel besylate is a novel salt form of Clopidogrel prepared to improve stability as well as safety of drug molecule. Aim of the study was to compare biopharmaceutical properties of Clopidogrel besylate and Clopidogrel bisulphate like solubility, permeability and dissolution.

EXPERIMENTAL METHODS

• Solubility determination

The solubility of the clopidogrel besylate and clopidogrel bisulphate substances was determined by saturated solution method in physiological pH range in the following media: pH 1.2 and pH 3.0 (diluted HCl), pH 4.5 (USP acetate buffer), pH 6.8 and pH 7.5 (adjusted HEPES buffers). Excess amount of drug was added in prescribed media in order to obtain saturated solutions. For each media three solutions were prepared and put on shaking water bath with temperature set on 37°C until equilibration was reached. After filtration or centrifugation the solutions were properly diluted and quantity dissolved was determined by HPLC detection.

• Permeability determination

Caco-2 cells (ECACC) were grown as previously described [3]. At passage 53, the cells were seeded on 12 mm Transwell® polycarbonate inserts with 3 μm pores at 60000 cells/cm^2 and grown for 20 days. The transport medium was HBSS-12mM HEPES pH 7.4. Clopidogrel bisulphate and Clopidogrel Besylate were prepared at 13 and 15 μg/ml respectively, which is equal to 31 μM of clopidogrel concentration. Propranolol and ranitidine were set at concentrations 100 μg/mL, while rhodamine at 1.9 μg/mL. pH of transport medium was set to 7.4. All solutions contained 1% DMSO. The transport was performed in apical to basolateral (AB) and in basolateral to apical (BA) direction. The P_{app} was calculated according to the equation:

$$P_{app} = (dQ/dt)/(A*C_0)\ (nm/s).$$

The quality of cell monolayers was monitored by measuring the TEER and rhodamine transport was determined to asses the P-gp expression.

• Dissolution testing

Samples developed using drug substances Clopidogrel bisulphate and Clopidogrel besylate in the same formulation were analyzed and compared with the product from the market containing Clopidogrel bisulphate as active drug substance. Dissolution testing was performed in 500 mL Fasted simulated gastric fluid (FaSSGF) pH 1.6 as dissolution medium using paddle apparatus, with rotational speed set at 50 rpm. Simulated gastric medium was chosen as appropriate due to pharmacokinetics data of Clopidogrel.

RESULTS AND DISCUSSION

Solubility of both salts, as it is shown in Figure 1, decreases as pH increases from 1.2 to 7.5. Solubility of clopidogrel bisulphate in acidic medium (pH 1.2) is significantly higher than solubility of clopidogrel besylate. Considering very high solubility of both tested salts in medium pH 1.2 this difference has no biological relevance since determined solubility of maximal dose (75 mg) is 200 times higher than dose/volume ratio. In other tested media no significant difference is observed.

Results of Clopidogrel permeability across Caco-2 cells are presented in Figure 2. The average P_{app} values in AB direction were about 16 * 10^6 cm/s. In comparison to the permeability standards propranolol and ranitidine, both clopidogrel salts are likely to be highly permeable. Permeability of rhodamine, P_{app}BA/P_{app} AB>10, demonstrated the expression of P-gp in Caco-2 cells. The BA/AB ratio between P_{app}'s for both clopidogrel salts was 0.6. The mass recovery values of clopidogrel measured during the transport, for all experiments were low, about 40-50 % for AB direction and 60 % for BA direction. The obtained low mass recovery can be attributed to the cell uptake or binding to the Transwell system.

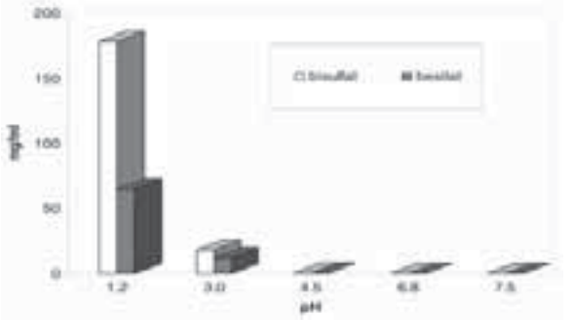

Figure 1: Comparison of Clopidogrel bisulphate and Clopidogrel besylate solubility in pH range 1.2 – 7.5.

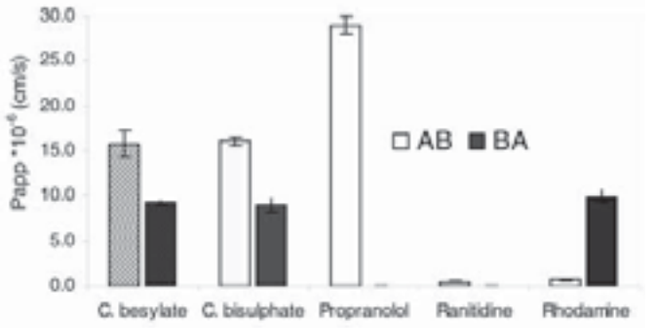

Figure 2: Results of permeability study of Clopidogrel salts and standards across Caco-2 cells.

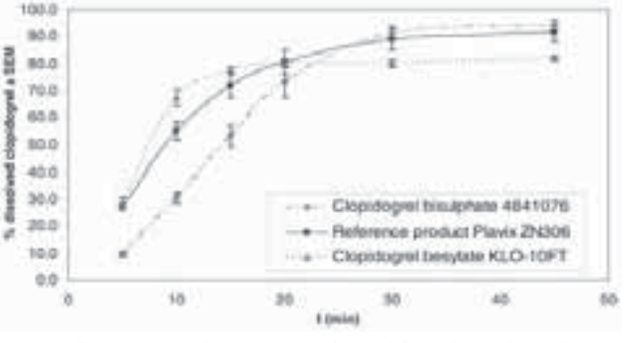

Figure 3: Comparative dissolution profiles of Clopidogrel bisulphate, Clopidogrel besylate and Plavix in FaSSGF, 500 mL, paddles, 50 rpm.

The results for permeability of clopidogrel salts are in accordance with reported high permeability of clopidogrel [2].

Dissolution results show, Figure 3, that Clopidogrel is faster released from formulation consists of Clopidogrel besylate salt than from formulation with bisulphate salt as well as product from the market. Therefore, additional formulation adjustment is needed in order to assure similar dissolution rate of both salts from formulation.

CONCLUSION

Both salts show pH-dependant solubility. According to BCS classification both salts, Clopidogrel bisulphate and Clopidogrel besylate are considered as low soluble. The permeability of clopidogrel besylate and clopidogrel bisulphate determined across Caco-2 cells is similar. Dissolution results show small difference in release rate between bisulphate and besylate salts which could be overcome with formulation adjustment.

References

1. Paulekuhn GS, Dressman J, Saal C, J. Med. Chem. 50:6665-6672 (2007).
2. Ki MH, Choi MH, Ahn KB, Kim BS, Im DS, Ahn SK Shin HJ. Arch Pharm Res. 31(2):250-8 (2008).
3. Khan MZ, Raušl D, Zanoški R, Zidar S, Mikulčić JH, Krizmanić L, Eškinja M, Mildner B, Knezević Z. Biol. Pharm. Bull. 27(10):1630-5 (2004).

PO073

Evaluation of dissolution profiles by the aid of a model of gastric emptying of tablets under fasting conditions

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INTRODUCTION

Gastrointestinal transit of solid oral dosage forms is most frequently evaluated by a non-invasive technique called gamma scintigraphy. Recently, oral dosage forms have become more and more sophisticated, meaning that the transit characteristics of these forms through the gastrointestinal tract might have an important impact on drug bioavailability and could, therefore, largely contribute to inter- and intraindividual variability of the pharmacokinetic properties of incorporated drug. The gastric emptying time of solid oral dosage forms is important for release and consequently for bioavailability of drugs. If drug release from the dosage form is pH dependent, then its release profile changes with gastric residence time.

Dissolution testing is frequently used as a tool for prediction of dosage form behaviour after its administration. To simulate *in vivo* situation the conditions in dissolution tests should be as close as possible to those *in vivo*. In case of single unit dosage forms (e.g. tablets) the gastric emptying is an all-or-nothing process. Consequently, the individual gastric emptying happens at a certain time point which is highly variable. On the other hand, gastric emptying of tablets in population might be represented by a relatively wide time interval. Time period during which tablets should be kept in simulated gastric fluid in *in vitro* dissolution tests is thus questionable.

The objective of this study was to develop a mathematical model that would adequately describe human gastric emptying of non-disintegrating tablets under fasting conditions of healthy subjects. On the basis of the model, it was aimed to assign the time periods during which tablets should be kept in simulated gastric fluid and generate a prediction of *in vivo* dissolution profile.

EXPERIMENTAL METHODS

Model of gastric emptying of tablets

A systematic literature search on studies of human gastric emptying of tablets under fasting conditions was undertaken in MEDLINE database. Special attention was paid to the following criteria: study design, tablet size and density, fluid intake with the tablets, method of assessment of gastric emptying and results. Individual gastric emptying times of non-disintegrating tablets administered with water were collected and ranked in ascending order taking ties into account. Proportion of tablets remaining in the stomach was calculated as $TR_t = [1\text{-rank}/n]_t$ where *rank* denotes the rank of an individual gastric emptying time and *n* is overall number of observations. Gastric emptying profile was plotted as proportion of tablets remaining in the stomach versus individual gastric emptying time. Four models (exponential, exponential with lag-time, Weibull and log-logistic) were proposed and fitted to constructed gastric emptying profile using nonlinear regression in the SPSS statistical program, version 15.0. The iterative Levenberg-Marquartd method was applied for the estimation of the model parameters. Akaike Information Criterion (AIC) was calculated for each model in order to select the most adequate one for describing the gastric emptying data. A lower value of AIC indicated a better fit.

The influence of tablet dimensions on gastric emptying in fasted volunteers was also examined. Round tablets were divided to small (less than 10 mm in diameter) and large (equal or more than 10 mm in diameter). Independent sample t-test was used for assessment of difference in gastric emptying rates of small and large tablets. Non-parametric Mann-Whitney test was used for assessment of differences in volume of water administered with tablets.

***In vitro* dissolution tests**

In vitro dissolution tests were performed on theophylline-containing cylindric dosage forms [1]. For dissolution testing flow-through method, developed at the Faculty of Pharmacy, University of Ljubljana, was used. Volume of the medium in 150mL beaker was 40 mL, temperature 37°C and flow rate 2 mL/min. Constant flow was maintained throughout the tests. The stirring rate (magnetic stirrer) was 100 rpm. Dosage forms were firstly exposed to simulated gastric fluid for 10, 40

or 120 min and then to simulated intestinal fluid. Residence times in artificial gastric juice were chosen on the basis of previously constructed gastric emptying profile. The amount of dissolved theophylline was measured by UV spectrophotometry at 271 nm.

RESULTS AND DISCUSSION

Weibull model (η = 70.2 min, β = 1.40) fitted the best gastric emptying profile. Gastric residence times (10, 40, and 120 min) were calculated as means of gastric emptying time intervals selected from the gastric emptying curve (Fig.1).

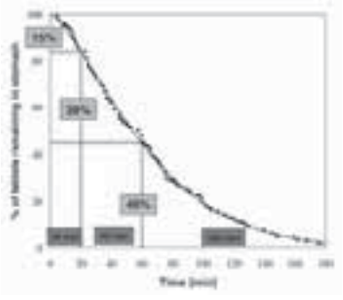

Figure 1: Gastric emptying of tablets (circles) and the Weibull model fit (solid line).

Statistical analysis and difference in mean gastric residence time (MGRT) showed that the gastric emptying of large tablets (n=59) was significantly faster than that of the small tablets (n=33). However, small tablets were administered with significantly smaller volume of water (mean 139 mL) than large tablets (mean 193 mL), what may also influence gastric emptying apart tablets size.

In vivo dissolution profile was predicted on the basis of *in vitro* dissolution profiles obtained at selected residence times in artificial gastric juice and the Weibull model of gastric emptying of tablets under fasting conditions (Fig.2). This was calculated as weighted average of *in vitro* dissolution profiles with proportion of tablets remaining in stomach representing the weights:

$$[\%D]_t = 0.15x[\%D(10\ min0]_t + 0.39x[\%D(40\ mni)]_t + 0.46x[\%D(120\ mni)]_t$$

where $[\%D(10min)]_t$, $[\%D(40min)]_t$ and $[\%D(120min)]_t$ denote % of theophylline dissolved at time t in dissolution tests where cylindric dosage forms were kept in simulated gastric fluid for 10, 40, and 120 min, respectively.

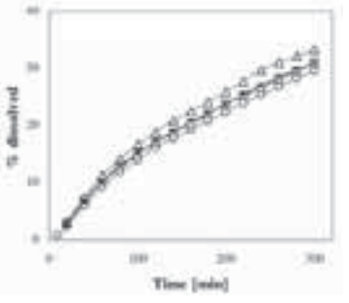

Figure 2: In vitro dissolution profiles at selected gastric residence times; 10 min (open circles), 40 min (squares), and 120 min (triangles), and predicted in vivo dissolution profile (filled circles).

CONCLUSION

We developed the method for prediction of *in vivo* dissolution profile on the basis of Weibull model of gastric emptying of tablets and *in vitro* dissolution tests which should be performed at selected gastric residence times. The method is best employed to the tablets with pH-dependent drug release for which *in vitro* dissolution profiles change with the time in which the tablet is kept in artificial gastric juice.

References

1. Quintavalle U, Voinovich D et al. EJPS 2008; 33:282–93.

PO074

The comparison of dissolution profile for ULCOSAN capsules 20 mg and ULCOSAN capsules 40 mg (Bosnalijek) with reference to LOSEC capsules 20 mg (AstraZeneca UK), LOSEC capsules 40 mg (AstraZeneca UK), LOSEC MUPS tablets 20 mg (AstraZeneca UK) and LOSEC MUPS tablets 40 mg (AstraZeneca UK)

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INTRODUCTION

Drug dissolution (or release) testing is an analytical technique used to assess release profiles of medicines in pharmaceutical products, generally solid oral products such as tablets and capsules (1). This test gains its significance from the fact that if a drug from a product is to produce its effect, it must be released from the product and should generally be dissolved in fluids of the gastrointestinal (GI) tract. Thusly, a drug dissolution test may be considered as an indicator of potential drug release and absorption characteristics of a product in humans as well as in animals (2). Dissolution testing is a required test currently used to demonstrate the performance of all solid oral dosage forms in which absorption of the drug is necessary for the product to exert a therapeutic

effect (3). The dissolution characteristics of the formulation are to be evaluated over the physiologic pH range of 1.2 to 7.5. The solubility of active ingredient(s) is one of the key aspects in the screening of possible dissolution media.

Omeprazole belongs to a class of antisecretory compounds, the substituted benzimidazoles, that do not exhibit anticholinergic or H2 histamine antagonistic properties, but that suppress gastric acid secretion by specific inhibition of the H+/K+ATPase enzyme system at the secretory surface of the gastric parietal cell (4). Absorption is rapid, with peak plasma levels of omeprazole occurring within 0.5 to 3.5 hours.

Since omeprazole and other PPIs (proton pump inhibitors) are all acid-labile, they must be protected from intragastric acid when given orally. This is achieved by the use of enteric-coated formulations, but differences in coating may influence protection against the acid and, consequently, may affect bioavailability (5). Omeprazole has low water solubility and is chemically very unstable in acidic media. This presents a significant challenge for pharmaceutical technologists as the ideal preparation must therefore protect the active ingredient against decomposition by gastric acid, as well as assure an immediate and complete release from the dosage form as soon as it has been transported to the site of absorption in the small intestine. To this end, omeprazole is currently formulated as delayed-release capsules and tablets containing enteric-coated pellets. Astra Zeneca has launched a tablet formulation of Losec (omeprazole) — Losec MUPS. MUPS stands for multiple unit pellet system and this technology has allowed the acid-protected omeprazole units contained in the capsule to be made into a tablet formulation, says the company. The new tablets work in the same way as Losec capsules, have the same licensed indications and equivalent efficacy and healing rates.(6)

During this in vitro trial, capsules' comparative dissolution profiles were made in comparison to capsules, as well as of capsules in comparison to MUPS tablets with the aim of determining and comparing dissolution of active ingredient – omeprazole – from two pharmaceutical forms. Obtained results of *in vitro* trials should help in forecasting *in vivo* absorption of omeprazole.

EXPERIMENTAL METHODS

In vitro dissolution (content release) of Omeprazole is performed according to general procedure USP< 711> apparatus 2, using rotating paddles method (1). Use 0.1mol/l hydrochloric acid, 900 ml as a first medium for 2 hours, and phosphate buffer pH 6.8, as a second medium for the next 45 min at the temperature of 37 °C ± 0.5 °C with mixing speed 100 rpm. Sample medium is to be taken every hour with first medium and after 10, 15, 25, 35 and 45 minutes with second medium, and assay is determined by spectrofotometric method.

Test parameters:

Apparatus 2	Method of rotating paddles
First medium	Hydrochloric acid 0.1 mol/l ; 900 ml
Second medium	Phosphate buffer pH 6.8 ; 900 ml
Temperature	37 °C ± 0.5 °C
Mixing Speed	100 rpm
Number of tested units	12

System for Dissolution	Varian Van Kel VK7010
Spectrophotometer	Varian Cary 50

RESULTS AND DISCUSSION

	f2
ULCOSAN capsules 20 mg (Bosnalijek) / LOSEC capsules 20 mg (AstraZeneca UK)	65.41
ULCOSAN capsules 20 mg (Bosnalijek) / LOSEC MUPS tablets 20 mg (AstraZeneca UK)	39.00
ULCOSAN capsules 40 mg (Bosnalijek) / LOSEC capsules 40 mg (AstraZeneca UK)	75.57
ULCOSAN capsules 40 mg (Bosnalijek) / LOSEC MUPS tablets 40 mg (AstraZeneca UK)	39.70
LOSEC MUPS tablets 40 mg (AstraZeneca UK) / LOSEC capsules 40 mg (AstraZeneca UK)	38.45
LOSEC MUPS tablets 20 mg (AstraZeneca UK) / LOSEC capsules 20 mg (AstraZeneca UK)	35.84

	f1
ULCOSAN capsules 20 mg (Bosnalijek) / LOSEC capsules 20 mg (AstraZeneca UK)	9,29
ULCOSAN capsules 20 mg (Bosnalijek) / LOSEC MUPS tablets 20 mg (AstraZeneca UK)	29,50
ULCOSAN capsules 40 mg (Bosnalijek) / LOSEC capsules 40 mg (AstraZeneca UK)	5,10
ULCOSAN capsules 40 mg (Bosnalijek) / LOSEC MUPS tablets 40 mg (AstraZeneca UK)	28,18
LOSEC MUPS tablets 40 mg (AstraZeneca UK) / LOSEC capsules 40 mg (AstraZeneca UK)	31,13
LOSEC MUPS tablets 20 mg (AstraZeneca UK) / LOSEC capsules 20 mg (AstraZeneca UK	35,79

CONCLUSION

According to obtained results, it can be concluded that ULCOSAN capsules 20 mg (Bosnalijek) and ULCOSAN capsules 40 mg (Bosnalijek) with reference to LOSEC capsules 20 mg (Astra Zeneca UK), LOSEC capsules 40 mg (Astra Zeneca UK) are similar, but ULCOSAN capsules 20 mg and ULCOSAN capsules 40 mg (Bosnalijek) with reference to LOSEC MUPS tablets 20 mg (Astra Zeneca UK), LOSEC MUPS tablets 40 mg (Astra Zeneca UK) are not similar.

Additionally, it can be concluded according to obtained results that LOSEC capsules containing 20 mg and 40 mg omeprazole are not similar to LOSEC MUPS tablets.

References

1. USP General Chapter on Dissolution <711>. United States Pharmacopeia and National Formulary; United States Pharmacopeial Convention, Inc.: Rockville,MD, 2005; pp 2412–2414
2. Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations; Guidance for Industry; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), U.S. Government Printing Office
   Washington, DC, 1997.
3. Gray VA, Brown CK, Dressman JB and Leeson J. 2001. A New General Information Chapter on Dissolution. Pharmacopeial Forum 27(6):3432-3439
4. Drugdex drug evaluations, 1974 - 2005 Thomson Micromedex, Micromedex(R) Healthcare Series Vol. 124
5. Vaz-da-Silva M, Loureiro A I., Nunes T, Maia J, Tavares S, Falcao A. et al. Bioavailability and Bioequivalence of Two Enteric-Coated Formulations of Omeprazole in Fasting and Fed Conditions, Clin Drug Invest 2005.
6. October 2, 1999 Vol 263 No 7065 p550 Products

PO075

The testing of the release characteristics from metoprolol tablets

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INTRODUCTION

Metoprolol is a drug that blocks the β adrenergic receptors, with a significant β1 adrenolitic effect and a weak quinidinic effect, lowering the cardiac frequency and blood flow. In small dosages, its action is selective, but in high dosages, used in the treatment of hypertension, it can affect the β2 adrenergic receptors, and the bronchial ways. Its clinical use is in treating mild and moderate hypertension.

In the present study we have manufactured tablets with in 50 mg and 100 mg of metoprolol by direct compression. After preparation, the resulting tablets we subjected to quality control tests, most important being the dissolution test.

We have determined "*in vitro*" the release rate of metoprolol from the tablets, in the conditions provided by the USP monography for this test, using 6 tablets and temperature and pH conditions similar to the human organism.

EXPERIMENTAL METHODS

We have formulated tablets with concentrations of 50 mgs and 100 mgs of metoprolol per tablet (table I).

As excipients, we have used Ludipress (custom-produced granules consisting of lactose monohydrate), Kollidon 30, and Kollidon CL (produced by BASF AG, Germany), Kollidon VA 64 (polyvinylpyrrolidone produced by BASF AG, Germany), sodium starch glycolate (produced by Cerestar GmbH, Germany), talcum and magnesia stearate (produced by Riedel-de-Haen AG, Germany). [1,2]

Table 1: The formulas of the 50 mg and 100 mg metoprolol tablets

Substances	Concentration mg/tablet		%		Function in formulation
Metoprolol	50	100	28.57	29.85	Active ingredient
Ludipress	110	212	62.86	63.28	Support granule
Kollidon VA 64	5	8	2.86	2.39	Binder, disintegrant
Sodium starch glycolate	4	6	2.28	1.79	Superdisintegrant
Talcum	3.5	5.5	2	1.64	Lubricant
Magnesia stearate	2.5	3.5	1.43	1.05	Lubricant
TOTAL	175	335	100	100	

All product components are perfectly compatible. The tablets were prepared by direct compression. [3]

RESULTS AND DISCUSSIONS

For both formulas, we have produced round, white coated tablets, with a lenticullar, smooth and uniform surface.

The experimental results of the quality determinations and of the assays performed on the tablets are shown in table II.

Table 2: The experimental results of the quality determinations and of the assays performed on the 50 and 100 mg metoprolol tablets

No.	Tested parameters	50 mg metoprolol tablets	100 mg metoprolol tablets
1.	Average weight, mg	174,8	334,6
2.	Disintegration time, min.	2,8	3,1
3.	Metoprolol assay, mg/tablet	49,1	98,7
4.	Friability, %	0,02	0,05
5.	Mechanical resistance, N	69	72

It can be observed that the tested characteristics for both types of tablets are optimal and within the admissible limits provided by official regulations. The tablets have a good mechanical resistance and a very low friability and an excelent disintegration time.

The experimental data from the dissolution test are shown in the following tables and figures. Table III shows the concentration values of six 50 mg metoprolol tablets recorded during the determination.

Table 3: The concentration values for the six 50 mg metoprolol tablets, in different moments in time

Parameter	Time (minutes)				
	5	10	15	20	30
Concentration %	61.58	84.89	90.14	94.86	99.36
Concentration %	65.22	86.73	91.23	95.66	99.99
Concentration %	63.96	86.12	91.02	95.54	99.87
Concentration %	62.11	85.39	90.97	95.15	99.26
Concentration %	61.87	85.07	90.68	95.03	99.35
Concentration %	62.49	86.26	91.19	95.26	99.92
Average value	62.87 17	85.74 33	90.87 17	95.25 00	99.62 50
Dispersion	1.4210	0.7333	0.4083	0.3042	0.3345
Disp/Average value	0.0226	0.0086	0.0045	0.0032	0.0034

After the first 5 minutes, the concentration of metoprolol varies from 61.58 % to 65.22 % and after 30 minutes it exceedes 99 %. The variation is nonlinear and can be considered exponential at a first approxia-

mation. This assumption is supported by the calculated values for the variation of the average concentration and of the average dispersion, shown in figures 1 and 2.

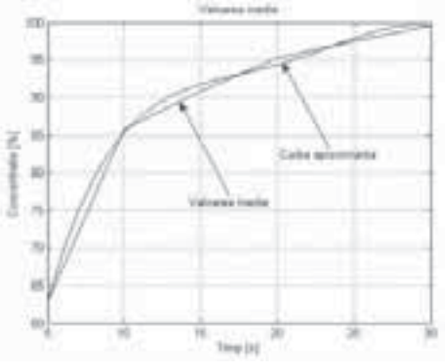

Figure 1: The variation of the average concentration for the 50 mg metoprolol tablets

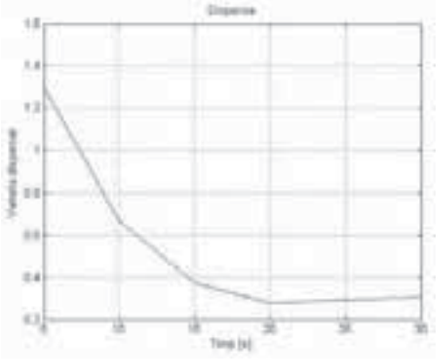

Figure 2: The variation of the dispersion for the 50 mg metoprolol tablets

Figure 3 shows the variation in time of the metoprolol concentration for the six 100 mg tablets. A similar variation can be observed, with small fluctuations from one tablet to the other. The charts shown in figures 3 and 4 are suggestive of the variation pattern of the average concentration and dispersion for the 100 mg tablets. An almost identical variation behaviour can be seen and an exponential shape.

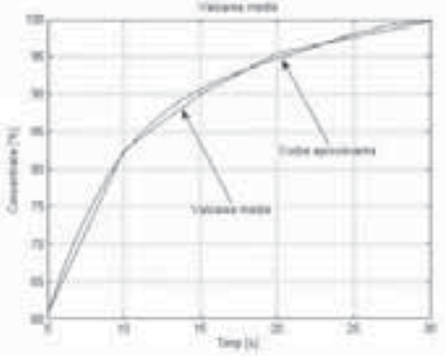

Figure 3: The variation of the average concentration for the 100 mg metoprolol tablets

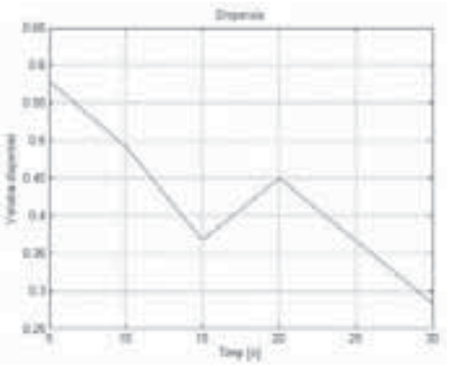

Figure 4: The variation of the dispersion for the 100 mg metoprolol tablets

By analysing the results, it can be concluded that the resulting values for concentrations are within the limits specified by the United States Pharmacopoeia, after 30 minute the tablets being dissolved in a 75 % proportion.

CONCLUSIONS

In the present study we have attempted the production of 50 mg and 10 mg metoprolol tablets, the formula containing, besides the active ingredient, other excipients: Ludipress, Kollidon VA 64, sodium starch glycolate, talcum and magnesia stearate. These excipients allow the direct compression, a method with certain advanatges over clasic tableting methods. The appearance of the dissolution curves are very similar for both types of tablets. The resulting experimental curves were modeled using polinomial and exponential equations.

References

1. Plaizier J.A., Vercammen, H.van den Bossche – Direct compression – Pharm.Ind.54 nr.11, 1992;
2. Heinz R, Wolf H, Schumann H, End L, Kolter K. *Formulation and development of tablets based on Ludipress and scale-up from laboratory to production scale.* 2000 May;26(5):513-21;
3. Krumme M, Schwabe L, Frömming KH. *Development of computerised procedures for the characterization of the tableting properties with eccentric machines. High precision displacement instrumentation for eccentric tablet machines.* Acta Pharm Hung. 1998;68:322-331.

PO076

Modelling of a particle size effect on dissolution of poor solubility API

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INTRODUCTION

Poor water solubility is attributed to almost half of the new molecular entities, synthesized annually by pharmaceutical companies [1]. For poor water solubility drugs solubility and dissolution rate often limit bioavailability and pharmacokinetic parameters, such as $C_{max,}$ t_{max} or AUC. Reduction of the particle size increases the dissolution rate and if the particles are in nano-meter range, the final solubility increases too.

The effect of particle size distribution (PSD) on the drug absorption is especially important for high-dose poor-solubility drugs. The model drug under study exhibits low aqueous solubility (0.2mg/ml). At the same time, the dose is high and more than 1 L of water would be needed to dissolve the whole dose. The drug exhibits high permeability and is very lipophilic. The drug is formulated as a tablet.

A limiting water solubility of the drug renders classical dissolution tests less selective, since only a fraction of the dose is dissolved in the dissolution volume. Consequently, only a fraction, dominated by smaller particles is studied in dissolution test and the effect of the biggest particles that carry the most material is obscured, but on the other side it can importantly affect bioavailability.

To have a usable tool for predicting dissolution behavior on the base of the whole particle size distribution, numerical model has been developed. It incorporates diffusion limited dissolution as well as a particle size effect on the final solubility of particles in nanometer range. The model is reversible and also covers crystal growth (but no nucleus formation) and mass transport processes such as the Ostwald ripening.

MATERIALS

The model drug was used in two different particle size distributions, prepared by micronization.

DISSOLUTION MODELLING

To study the effect of the particle size distribution on dissolution, numerical model has been developed. The dissolution of particles takes place in a compartment of an aqueous environment and is solubility/diffusion limited. The secondary process is a transport from the primary compartment to a second-reservoir compartment, where it is completely bound to plasma proteins, so that effective concentration gradient across the membrane only depends on the concentration in compartment A (Schematically presented in Figure 1). The amount of the drug in the second compartment is at last reduced by 1.st order elimination.

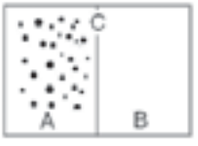

Figure 1: Dissolution of particles takes place in aqueous compartment A, then transported accross membrane C to compartment B where it is accumulated.

Dissolution is diffusion controlled and the mass transport is described by the diffusion equation (in this case for a spherical particle). The equilibrium solubility depends on the particle size via the Kelvin equation.

$$j = \frac{D}{l} 4\pi r^2 (c_0 e^{\frac{p_v}{RTr}} - c)$$

Here **j** is the mass current, **D** diffusion constant, **l** diffusion length, **r** is a particle radius, $\mathbf{c_0}$ is a final saturated concentration, **γ** is a surface energy, $\mathbf{v_v}$ molar volume, **R** gas constant, **T** temperature and **c** the actual concentration in the solution. The equation is rewritten in non-dimensional form and integrated in time.

In the model the number of particles in a single bin of PSD is conserved, except the number of particles in the lowest bin, where in each integration step, particle size is reduced according to the shrinkage of the lowest present bin.

In each integration step, the material loss or gain (during crystallization from an over-saturated solution) is calculated for each bin in PSD and accordingly the lower and upper bin limits are reduced or increased respectively, reflecting the dissolution or growth of particles.

Since particle size distribution and final solubility are determined independently and dissolution volume is known, only two independent free parameters enter into dissolution model for compartment A: one for the time (bound to diffusion constant and diffusion length) and second the Kelvin radius, which determines solubility dependence on particle radius: $r_K = \gamma v_v/RT$. This parameters are fitted to a dissolution profile of a drug with known PSD and the dissolution profile of any PSD can be calculated.

For calculation of the in-vivo effect of PSD the model has been extended and two additional parameters are added. One determines the permeability through the membrane and can be determined via permeability studies or PBE study, the other is elimination time and can often be found in literature for known drugs.

RESULTS

Two formulations with different particle size distributions were manufactured. PSDs are shown in Figure 2.

The effect of PSD change on dissolution is not so obvious, since no total dissolution of drug takes place in a dissolution volume (950ml). On the other hand, the simulation indicates the growth of larger undissolved particles in a wider distribution of prep. 2. This grown particles contribute

to a delay at the end stage of in-vivo dissolution, shown in extended dissolution model in . Especially C_{max} is affected strongly.

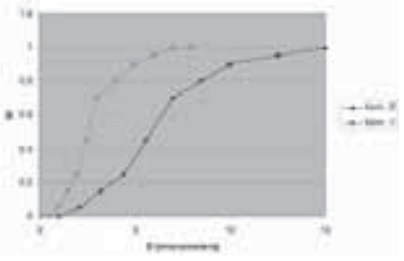

Figure 2: Cumulative particle size distributions for preparation 1 and 2.

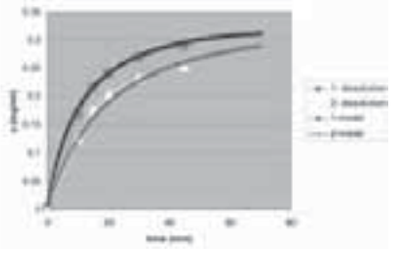

Figure 3: The model was fitted to a known dissolution of preparation 1. Dissolution of preparation 2 with different PSD is calculated with the same parameters and then compared to performed dissolution test.

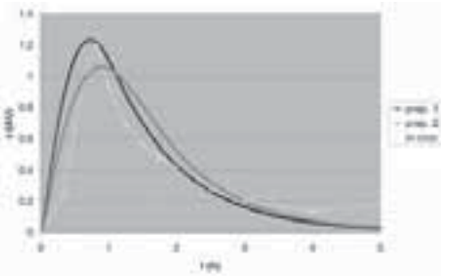

Figure 4: Modelled in-vivo concentration profiles, based on PSD dependent dissolution model. Compared to in-vivo determined drug concentration profile (yelow).

CONCLUSION

Numerical modeling of dissolution can be an useful tool for the evaluation of an effect of the PSD on dissolution and on an in-vivo drug concentration profile. At the same time, the model can be used to study mass transport processes in suspension, for example Ostwald ripening.

References

1. R. Saffie-Siebert et. al. Drug Discovery World Summer 2005, p.71-76

PO077

Supercritical fluid particle design for increasing dissolution rate of poorly-soluble active pharmaceutical ingredients

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ABSTRACT

We intend to present some of our recent results on the dissolution rate enhancement of poorly-soluble APIs by using supercritical fluids (SCF) in order to micronize these compounds, or to formulate them by co-precipitation or micro-encapsulation. Two Supercritical Fluid particle design processes were assessed: Rapid Expansion of Supercritical Solutions (RESS) and Fluid-Assisted Micro-Encapsulation (FAME).

In many cases, SCF-micronization of neat APIs led to drug nano-/microparticles with a poor processability that hindered the benefit of micronization. A process that permits to take profit of the specific surface area increase of SCF-micronized particles will be presented: the micronized particles generated during the micronization process are collected on a water-soluble excipient bed. Micronized drug particles are dispersed on the surface of the excipient (Fig. 1), facilitating the access to the surface for dissolution, and downward processing of the particles immobilised on a free-flowing powder.

SCF are also particularly valuable to produce composite particles exhibiting enhanced processability and dissolution rate performance. Recent results from *in vitro* dissolution rate measurements showed that solid dispersions produced by SCF processes, incorporating hydrophilic or amphiphilic polymers, made it possible to substantially increase the dissolution performance of poorly soluble drugs.

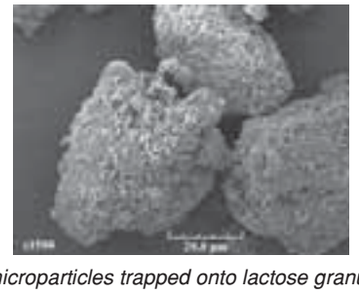

Figure 1: API microparticles trapped onto lactose granules

PO078

The effect of additives on solubility and permeability of dexamethasone-cyclodextrin eye drop microsuspension

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INTRODUCTION

Corticosteroid containing eye drops are standard anti-inflammatory therapy after cataract extraction and IOL implantation to suppress the acute postoperative inflammatory caused by surgical trauma. Dexamethasone (Dx) is one of the most frequently used topical ocular corticosteroids. Topical corticosteroids are used for diseases of the outer eye and anterior segment of the eye [1].

Cyclodextrins (CDs) are water-soluble oligosaccharides that solubilize poorly soluble ophthalmic drugs through complexation. CDs act as true carriers by keeping the drug molecules in solution and delivering them to the surface of eye where they partition into the eye [2].

Various approaches have been undertaken to increase the precorneal residence time of topically applied drugs. For example, addition of polymers will increase viscosity of the drug formulation, delaying the drug's washout from the tear film and increase its bioavailability [3].

The aim of this study is investigation of the effect of additives on solubility and permeability of Dx from suspensions by using CD mixtures as carriers.

EXPERIMENTAL METHODS

• Phase solubility profiles

The solubility of Dx in water or aqueous CD solutions was determined by a heating method [4]. Excess amount of the Dx was added to a solution containing 0 to 20% (w/v) CD, with benzalkonium chloride (BAC) (0.02%), EDTA (0.1%) or hydroxypropyl methylcellulose (HPMC) (0.1%), individual compounds or mixtures thereof. The filtrate was diluted and analysed by HPLC [4]. Phase solubility studies were conducted with and without the addition of additives. γCD, HPγCD and mixtures thereof were investigated. The complexation efficiency (CE) was determined from the linear phase-solubility diagram (a plot of the total drug solubility ($[Dx]_t$) versus total CD concentration ($[CD]_t$) in moles per liter):

$$CE = \frac{\text{Slope}}{1-\text{Slope}} = \frac{[\text{dexamethasone/CD}]}{[CD]} = K_{1:1} \cdot S_0$$

• Effect of additives on Dx solubility and permeability

The 1.5% (w/v) Dx eye drop suspensions were prepared by dissolving 150 mg of Dx in 9 ml of an aqueous solution containing BAC (2 mg), EDTA (10 mg) and various type and amount of cyclodextrin (γCD, HPγCD and mixtures of γCD/HPγCD). HPMC (0.1-0.75% w/v) was added to the formulation and pH adjusted to 7.4 with NaOH. Osmolality was adjusted to 260-330 mOsm/kg with NaCl. The permeability studies of Dx eye drop preparations were carried out using Franz diffusion cell apparatus. The donor chamber and the receptor compartment were separated with a cellophane membrane MWCO 3500. The receptor phase was PBS pH 7.4 containing 5% (w/v) γCD/HPγCD (50/50). The study conducted at room temperature (22-23°C). The flux and apparent permeability coefficient (P_{app}) was calculated from the linear slope (dq/dt) of each permeability profile and the Dx concentration in the receptor phase determined by HPLC (Eq.2).

$$J = \frac{dq}{A \cdot dt} = P_{app} \cdot C_d$$

RESULTS AND DISCUSSION

Table 1 presents the CE ratio calculated from phase solubility profile using non-additive eye drop formulations as a reference. The CE of Dx in HPγCD and in γCD/HPγCD (ratios 80/20 and 20/80) containing each of additives tested were lower than, and not different from those, of non-additive formulations. This could be due to the presence of second guest molecule, i.e. due to competitive effect. However, when adding the combination of EDTA, BAC and HPMC to the formulation containing γCD/HPγCD (80/20), it improves complexation. Interestingly, the CE ratio of Dx in γCD solution containing BAC was significantly higher than that non-additive formulation. This could be due to the quaternary structure of BAC which acts as a surface active agent solubilizing insoluble Dx/γCD complexes. Addition of HPMC, a water-soluble polymer known to solubilize aggregated drug/CD complexes, resulted in the highest CE ratio.

Table 1: Effect of additives on Dx CE ratio using the non-additive formulations as a reference.

The additives	γCD	HPγCD	γCD/HPγCD	
			(80/20)	(20/80)
No additive	1.000	1.000	1.000	1.000
EDTA	0.881	1.152	0.847	0.805
BAC	1.569	1.136	1.161	0.853
EDTA+BAC	0.956	0.887	0.936	0.687
HPMC	1.251	1.114	1.004	0.709
EDTA+BAC+HPMC	1.954	1.175	1.415	0.978

The influence of HPMC concentration on CE of Dx was not due to solubilization of Dx by HPMC in the HPγCD and γCD/HPγCD formulations (Fig. 1). However, at higher concentrations HPMC appears to form ternary complex with Dx/γCD complexes resulting in enhanced Dx solubility. In such cases the polymer forms non-inclusion complexes by interacting with OH-groups of the Dx/HPγCD complex aggregates.

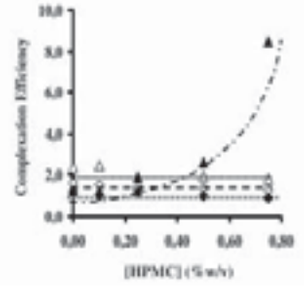

Figure 1: CE of Dx in CD solution containing different HPMC concentrations; ▲: γCD; □: γCD/HPγCD (ratio 80:20); ○: (HPγCD); ◇: γCD/HPγCD (ratio 20:80)

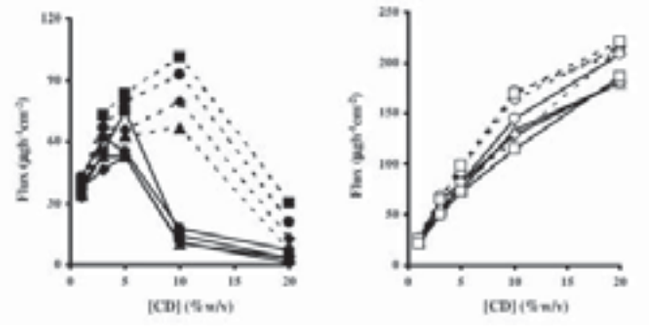

Figure 2: The effect of additives in aqueous CD solution on the flux of Dx through semi-permeable cellophane membrane MWCO 3500; □○: EDTA; ▲□: BAC; ●◇ : HPMC; ◇□ all additives; filled symbol with solid line: γCD; filled symbol with dotted line: γCD/HPγCD (ratio 80:20); opened symbol with solid line: HPγCD; opened symbol with dotted line: γCD/HPγCD (ratio 20:80)

Any further increase in the concentration of HPMC, did not enhance Dx permeability that is in agreement with previous observations by Loftsson T., et.al [5]. Therefore, it was decided to keep the HPMC conc. at 0.10% in the Dx suspensions. Fig. 2 shows flux of Dx from γCD, HPγCD or their mixtures, with either additive individual compounds or mixtures. The additives did not affect the Dx flux through membrane. However, when small amount of γCD was added to the HPγCD solution, the flux was increased due to consequent increase in total amount of dissolved Dx. A large flux increased was observed from formulations composed of 10% of γCD/HPγCD (80/20) containing all the additives in a mixture, compared to that of γCD. It showed optimum flux (66-101 mg h^{-1} cm^{-2}) and permeability coefficient (3.4-3.8x10^{6} cm s^{-1}). Addition small amount of HPγCD in γCD solution solubilized the aggregate species of Dx/CD complexes. This formulation is now being developed further as an eye drop vehicle.

CONCLUSIONS

Addition of BAC, a surface active agent, and HPMC, a water-soluble polymer, did enhance the complexation. The solubility of Dx in γCD suspensions increased with increasing HPMC conc. Addition of HPγCD to Dx/γCD complex suspensions did enhance Dx flux through a semi-permeable membrane.

REFERENCES

1. Kristinsson, J.K. et. al (1996) *IVOS* **37**(6), 1999-1203.
2. Loftsson, T. and Järvinen, T. (1999) *Adv. Drug Delivery Revs.* **36**. 59-79.
3. Koevary, S.B. (2003) *Curr. Drug Met.* **4**. 213-222
4. Loftsson, T., Hreinsdóttir, D., Másson, M. (2005*) Int. J. Pharm.* **302**, 18-28.
5. Loftsson, T., Másson, M., Sigurdsson, H.H. (2002*) Int. J. Pharm.* **232**, 35-43

PO79

pH-surfactant mediated solubility and dissolution of poorly water-soluble drugs: mathematical modeling

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INTRODUCTION

According to Biopharmaceutics Classification System (BCS), solubility and, consequently, dissolution are the most critical factors influencing oral absorption of BCS Class II drugs. However, several investigations [1,2] pointed out that, depending on the administrated dose, some of these drugs dissolve almost completely *in vivo*, meaning that solubility in the gastrointestinal tract (GIT) might not limite their absorption.

The objective of this study was to determine whether certain BCS Class II drugs can be solubilized by the combined effect of pH and surfactant and, accordingly, define appropriate, physiologically relevant pH-surfactant combination for their *in vitro* testing. An equilibrium-based mathematical model (EBMM) [3] was used to describe the pH-surfactant mediated solubilization of three model compounds: glimepiride (GLM) (pKa 6.2) and gliclazide (GLK) (pKa 5.8) as weak acids with no dissolution test requirements in USP or other regulatory documents and carbamazepine (CBZ) (pKa 14). USP XXII (1990) incorporated dissolution test for CBZ, but certain discrepancies between *in vitro* and *in vivo* data indicate that finding a suitable dissolution medium for this drug is still an

issue. Sucrose-laurate [4] was used as a surrogate for naturally occurring surfactants.

EXPERIMENTAL

• Materials

Carbamazepine (Freedom Chemical Diamalt GmbH, Germany), gliclazide (Zhejing Jiuzsou Pharmacy, China) and glimepiride (Zydus Cadila, India) were used as received. Sucrose laurate (Surfhope® SE Pharma, D-1216) was a gift from Mitsubishi-Kagaku Foods Corporation. All other chemicals used were of analytical grade.

• Solubility study

Equilibrium solubility was determined by a "shake-flask" method. Media employed were 0,2M KH_2PO_4/0,2M NaOH buffers pH 4.5 and pH 9.0 with/without the addition of 0.5% and 1% D-1216. The amount of drug dissolved was determined by UV analysis at the wavelength of the relative maximum absorption.

• Intrinsic dissolution rate

The intrinsic dissolution tests were performed using modified USP apparatus type II. Drug powder (500mg) was compressed in a stainless steel die to form a circular compact, with a radius of 12mm. The die was placed on the bottom of the dissolution vessel, so that the single face of the compact was exposed to 900ml of the dissolution media maintained at 37 ± 0.5°C. Rotational speed was adjusted to 75rpm. Dissolved amounts of the tested drugs were determined at suitable time intervals (within six hours) using UV analysis.

• Theoretical basis

EBMM assume that total drug solubility can be expressed as a sum of the solubilities of different species coexisting in a solution. For weak acids, such as GLM and GLK, unionized and ionized form in solution along with their corresponding micellar forms contribute to total solubility. In the case of non-ionizable compound (CBZ), the model is simplified concerning that only unionized form, as a free drug and in micelles, is present at the applied pH conditions.

Following total solubility concept, total flux of the drug can be expressed as a sum of dissolution rates of individual species. Assuming that micellar solubilization coefficients for unionized (Kn) and ionized (Ki) form obtained from the solubility study, along with diffusitivity values calculated from intrinsic dissolution rate measurements are constant under tested experimental conditions, these values can finally be used to estimate drug solubility/dissolution at various pH/surfactant concentration systems.

RESULTS AND DISCUSSION

Both solubility and dissolution of carbamazepine linearly increased with the addition of surfactant (Table 1 and Fig. 1) nevertheless of pH value indicating that pH change does not have an impact on CBZ solubility and dissolution, as expected according to its pKa value.

Glimepiride showed both pH and surfactant dependent solubility/dissolution, although it was evident that pH had higher effect on total solubility/dissolution enhancement than the presence of surfactant. Addition of surfactant enhanced solubility even further since both unionized and ionized drug were solubilized by micelles, especially at higher surfactant concentration. The obtained data are presented in Table 1 and Fig. 2.

Table 1: Equilibrium solubility of carbamazepine and glimepiride in pH/D1216 systems

	CBZ solubility (mg/ml)	GLM solubility (μg/ml)	
surfactant concentration	pH 9.0	pH 4.5	pH 9.0
0%	0.230	2.04	47.44
0.5% (w/v) D1216	0.479	2.66	91.19
1% (w/v) D1216	0.972	64.21	328.60

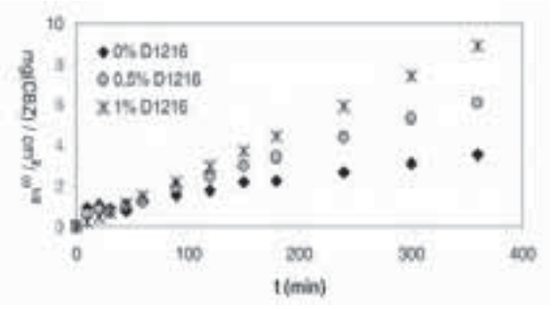

Figure 1: Intrinsic dissolution rate of carbamazepine in pH 9.0 with/without D1216

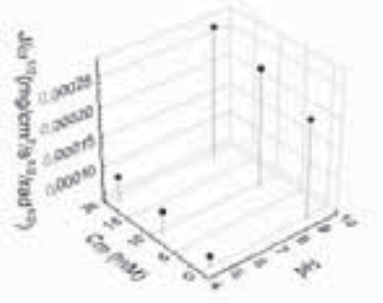

Figure 2: Intrinsic dissolution rate of glimepiride as a function of pH and D1216 concentration

Considering the highest dose strength of CBZ IR tablets (400mg) and GLM (4mg), calculated dose numbers ($D_{0(CBZ)}$=1.00 in pH 7.4 with 2% D-1216; $D_{0(GLM)}$=0.64 in pH 7.4 with 0.5% D-1216) indicate that optimal solubilities can be achieved in physiologically relevant conditions and, therefore, proposed media could further be utilized for dissolution testing of these particular drugs.

Both in the case of CBZ and GLM, the less significantly increased dissolution rate relative to solubility was due to smaller diffusion coefficients of the drug-loaded micelles (Table 2).

Table 2: Diffusivity coefficients for free and micellar forms of carbamazepine and glimepiride

		diffusion coefficients [cm^2/s]	
		free drug	drug in micelle
CBZ	unionized form	$1.33\ 10^{-5}$	$4.68\ 10^{-6}$
GLM	unionized form	$5.73\ 10^{-4}$	$7.97\ 10^{-7}$
	ionized form	$1.78\ 10^{-5}$	$2.31\ 10^{-7}$

Solubility studies of gliclazide revealed a deviation from Henderson-Hasselbalch equation, which resulted as a limitation for mathematical modeling.

CONCLUSION

The results obtained support the idea that optimal conditions for biorelevant *in vitro* testing of some poorly water-soluble drugs can be predicted by mathematical modeling. The proposed model could also be used to evaluate whether dissolution is expected to be a rate-limiting step in the overall absorption of some BCS Class II drugs. However, because the model is based on log-linear relationship, its application for the systems to which the log-linear model is not applicable, may be questionable.

References

1. Sheng J. et al. Eur J Pharm Sci 29: 306-314 (2006).
2. Yazdanian M. et al. Pharm Res 21: 293-299 (2004).
3. Jinno J. et al. J Pharm Sci 89: 268-274 (2000).
4. Grbic S. et al. 5th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Geneva, 2006

PO080

Preparation of an amorphous drug by melt-extrusion

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INTRODUCTION

Enhancements of the water solubility of drugs, classified in the BCS II or BCS IV, is one of the major interests of papers published recently[1-3]. In the case of drugs of BCS II biovailability depends mainly on their solubility rate, while in BCS IV poor membrane transport is an additional limiting factor.

Possibilities available to improve the water solubility of such drugs are associated with chemical or physical modifications as follows: Chemical control through:

- formation prodrug of good water solubility if its metabolite provides the expected biological response.
- formation salt of the API, having better water solubility than the original molecule, if its chemical character allows reaction with bases or acids.
- Inclusion of polar group if it does not affect the bioactivity of the molecule.

Physical control of:

- particle size: micronization, nanonization,
- morphology: habit of crystals, polymorphs, amorphization,
- complexes, solubilization: cyclodextrines, surface active molecules,
- drug dispersion in matrices: eutectic mixtures, solid fine dispersion
- molecular dispersion, solid solution

Solid fine dispersion and solid solution can be prepared by solution or melt method. The first one requires a solvent to dissolve the carrier and the drug together sufficiently then the solvent will be eliminated by spray drying, freeze drying, evaporating etc. Melt technologies are environment friendly and more productive than the solvent applying ones [1].

Melt-extrusion is widely used in the plastic and food industry and its application in the pharmaceutical technology, for forming granules, pellets and even tablets, is growing rapidly because dispersion can be controlled efficiently. Solid fine dispersion and/or solution can be prepared by melt-extrusion if an appropriate polymer is found.

Such polymer is expected to have a melting point close to the API, low viscosity at the temperature of blending and rigidity after the extrusion to allow further processing such as spheronization, grinding, tableting [1-3].

The aim of this work was to prepare amorphous spironolactone (SPIR) in form of inclusion complex with hydroxy-propyl- -cyclodextrin by melt-extrusion. First the molecular ratio of API to HPCD necessery for the entire complexation was determined in solvent evaporated samples by DSC and Raman-spectroscopy. It was followed by the preparation of extrudates with different matrices and parameters for sufficient complexation detected by Raman-spectroscopy.

EXPERIMENTAL METHODS:

• Solvent evaporation

The samples containing different molecular ratio of SPIR and HPCD were prepared by solvent evaporation using a Laborata 4000 type Heidolph apparatus.

• Melt-extrusion

Extrudation procedures were carried out in HAAKE Rheomax CTW5 MiniLab and Collin ZK 25 T extruders. Both machines are co-rotating twin screw extruders.

• Thermal analysis

The melting points of the materials and the temperature limit of their stability was determined using DSC 92 module of Setaram.

• Evaluation of extrudates

The complexation in extrudates was investigated by micro-Raman mapping using a LabRam type Jobin Yvon apparatus.

RESULTS AND DISCUSSION:

• Evaluation of solvent evaporated samples

Before melt-extrusion the melting/decomposition characteristics of the complexes with different molar ratio have been determined by DSC method (Fig. 1).

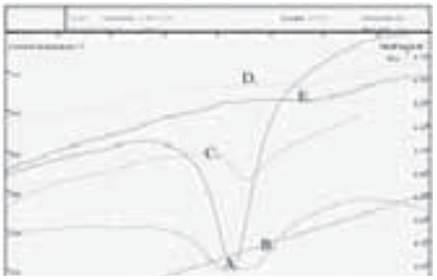

Figure 1: DSC curve of the SPIR-HPCD systems, (A) physical mixture and solvent evaporated complexes with molar ratio: (B)2:1; (C) 1:1; (D) 1:1,5; (E) 1:2

DSC results indicate the entire complexation at molar ratio of 1:1,5.

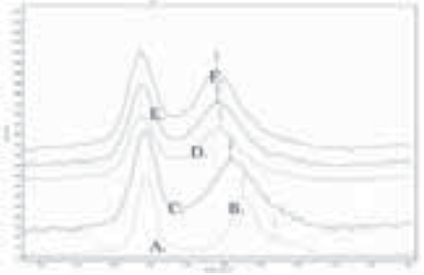

Figure 2: Raman spectras of the solvent evaporated samples: (A) pure HPCD; (B) pure spir; (C)2:1; (D) 1:1; (E) 1:1,5; (F) 2:1

The characteristic Raman peak of SPIR was shifted and became broader in the presence of HPCD. It is in coincidence with the results of DSC measurements.

• Evaluation of complexation in extrudates

Fig 2 shows the Raman-map of the extrudate containing SPIR dispersed in starch/sorbitol matrix processed at 110°C. As it is shown by the grey colour the melt-extrusion resulted in mostly fine dispersion of API but some aggregates could be detected as well.

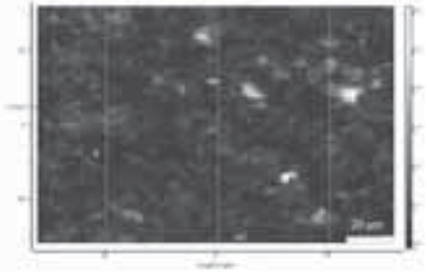

Figure 3: Micro-Raman chemical map of the melt-extrudated product. Black colour means the matrix, grey is the finely dispersed API and white is the aggregated API.

Micro-Raman mapping proved to be a convenient technique to characterise the state of dispersion and additionally to the DSC it could be applied to confirm the changes in the crystalline phase.

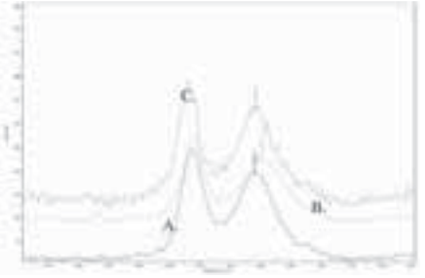

Figure 4: Raman spectras of (A) solvent evaporated reference containing SPIR and HPCD in molar ratio 1:1,5; and SPIR-HPCD containing extrudates in (B) Starch matrix; (C) Starch/sorbitol matrix

The Raman spectras of the solvent evaporated samples was used as reference for the evaluation of complexation in extrudates. Fig 4 indicates that in the starch/sorbitol system a higher complexation rate was achieved.

CONCLUSION

Melt-extrusion proved to be efficient granulation technique to improve the homogeneity and degree of dispersion and enhancing this way the water solubility of the investigated drug. Furthermore in the presence of HPCD the fine dispersion turns to a molecular dispersion also known as solid solution. Chemical mapping made by Micro-Raman method was applicable to detect the degree of dispersion and to distinguish the different polymorphs.

ACKNOWLEDGMENT

Our work was supported by BUTE Natural and Sport Scientific Association and Hungarian Research Found OTKA TO49121.

References

1. Christian Leuner, Jennifer Dressman: Improving drug solubility for oral delivery using solid dispersions, European Journal of Pharmaceutics and Biopharmaceutics, 50 (2000) 47-60
2. Jörg Breitenbach: Melt extrusion: from process to drug delivery technology, European Journal of Pharmaceutics and Biopharmaceutics, 54 (2002) 107–117
3. Michael M. Crowley, Feng Zhang, Michael A. Repka, Sridhar Thumma, Sampada B. Upadhye, Sunil Kumar Battu, James W. McGinity, Charles Martin: Pharmaceutical applications of hot-melt extrusion: Part I., Drug Development and Industrial Pharmacy, 33 (2007) 909-926
4. Osama Abd Elazeem Soliman, Kenya Kimura, Fumitoshi Hirayama, Kaneto Uekama, Hassan Mohamed El-Sabbagh, Abd El-Gawad Helmy Abd El-Gawad, Fahima Mahmoud Hashim: Amorphous spironolactone-hydroxypropylated cyclodextrin complexes with superior dissolution and oral bioavailability, International Journal of Pharmaceutics, 149 (1997) 73 - 83
5. Omid Rajabi, Farnoosh Tayyari, Roshanak Salari, Sayyed Faramarz Tayyari: Study of interaction of spironolactone with hydroxypropyl-b-cyclodextrin in aqueous solution and in solid state, Journal of Molecular Structure, accepted 30 July 2007

PO081

Dissolution improvement of high drug-loaded solid dispersion of Gelucire 44/14

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ABSTRACT

The objective of the study was an investigation of a high drug-loaded solid dispersion system consisting of drug, carrier, and surfactant. Solid dispersions of a water-insoluble drug oxcarbazepine (OXC) with Gelucire 44/14, (a saturated polyglycolized glyceride) namely binary solid dispersion systems were prepared at drug to carrier not less than 5:5 using solvent method. Polysorbate 80, a nonionic surfactant, was incorporated into the binary solid dispersion systems as the third component to obtain the ternary solid dispersion systems. The powder x-ray diffraction and differential scanning calorimetric studies indicated that crystalline OXC existed in the solid dispersions with high drug loading. However, from SEM it was observed that a decreased crystallinity of the solid dispersions obtained revealed that a portion of OXC was in an amorphous state. The results indicated a remarkably improved dissolution of drug from the ternary solid dispersion systems when compared with the binary solid dispersion systems. This was because of polysorbate 80, which improved wettability and solubilized the non–molecularly dispersed or crystalline fraction of OXC. So, it was concluded that the significant high drug-dissolution rate was achieved by a ternary solid dispersion system using Tween 80 as a third component and the fastest drug dissolution was obtained from a ternary solid dispersion containing OXC/Gelucire 44/14/Tween 80 of 5:5:1 wt/wt/wt.

METHODS

High drug-loaded solid dispersion preparation

OXC solid dispersions of both binary and ternary systems were prepared by conventional solvent method using Gelucire 44/14 as a carrier. In the binary system, the solid dispersions of 5:5 and 7:3 wt/wt of drug to carrier were prepared. The mixture of drug and carrier was dissolved in methanol. In the ternary system, the surfactant was added into the solution of drug and carrier to obtain the final weight ratio of drug/carrier/surfactant of 5:5:1 and 7:3:1 wt/wt/wt. The solvent was evaporated under reduced pressure using a vacuum dryer at 40^0C until complete evaporation. To ensure the residual solvent was completely removed, the solid mass was further dried in a vacuum oven at room temperature for 24 to 48 hours or a constant weight was obtained. The resulting solid was pulverized and sieved. The particle fraction of 70 to 200 μm was used in the experiment. The physical mixtures of binary system containing drug to carrier of 7:3 wt/wt were prepared by manually mixing the appropriate amount of 70- to 200- μm particle size fractions of OXC and carrier. In ternary physical mixtures, Tween 80 was gently mixed to the mixture of binary drug-carrier system to yield drug/carrier/Tween 80 of 7:3:1 wt/wt/wt.

CHARACTERIZATION

Powder X-ray diffraction, Differential scanning calorimetry, Scanning Electron Microscopy, Dissolution study

CONCLUSION

The high drug-loaded OXC binary solid dispersion with Gelucire 44/14 exhibited faster but not obviously in drug dissolution characteristics than the intact OXC. The significant high drug-dissolution rate was achieved by a ternary solid dispersion system using Tween 80 as a third component. In the ternary system, the effect of the Drug and carrier ratio was obviously seen. The same drug and carrier ratio, the higher the dissolution rate. Tween 80 played an important role in drug dissolution from the ternary system by improving drug molecular dispersion in the polymer matrix as well as drug wettability and solubility. The fastest drug dissolution was obtained from a ternary solid dispersion containing OXC/Gelucire 44/14/Tween 80 of 5:5:1 wt/wt/wt.

PO082

Combined solubilizing effect of pH, co-solvents and surfactants for the formulation of a parenteral solution containing miconazole

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INTODUCTION

Poor water solubility of active pharmaceutical ingredients is a great issue in pharmaceutical development. Unfortunately a wide range of chemicals exhibit inadequate solubility. This issue is prominently important in formulating a liquid dosage form, because in such cases ensuring the therapeutically effective amount of the ingredient is a great practical problem. Consequently, formulating water insoluble or poorly soluble drugs in liquid dosage forms has been met by many scientists and several methods of preparing parenteral medications of poorly soluble chemicals are known in the art. Some of these techniques include formulations of liposomes, emulsions, microemulsions, nanoemulsions or complex formation with cyclodextrins, hydrotropic solubilization, and formation of prodrugs. Other than these rather complicated approaches to increasing solubility, more conventional ways include adjusting the pH of the solution, using organic co-solvents or micellization [1,2].

Generally systemic mycoses are chronic and develop slowly as in the case of coccidioidomycosis, where primarily an acute respiratory disease develops followed by a chronic, often fatal infection of the skin, lymph glands, spleen and the liver. Other fungi may infect the kidney, liver, spleen, heart, eyes, brain, prostate, bone and may cause other infections. Whatever the case, mycoses are severe diseases and are to be dealt with.

A group of active pharmaceutical ingredients mainly built up of a five-membered nitrogen heterocyclic ring containing at least one other non-carbon atom, selected from the group of nitrogen, sulfur and oxygen, and contain side chains, the azoles have been shown to be effective agents against various fungi. Their fungicidal and fungistatic effects can be credited to their ability to inhibit sterol 14- -demethylase, a microsomal cytochrome P450 – dependent enzyme system. Azoles were shown to be effective against Blastomyces, Histoplasma, Coccidioides, Aspergillus and Candida, and other significant fungi including Cryptococcus, Torulopsis, Paracoccidioides, Rhizopus and Mucorand. These fungi are the most commonly recovered from patients suffering from such microbial infections [3].

As stated above, azoles are a group of antifungal agents that can be used in the treatment of systemic fungal infections. Although these compounds are potent molecules, most of them show poor water solubility, thus formulation of a composition, which comprises therapeutically effective quantities of active agent, is difficult. One way of overcoming this issue was with the use of Cremophor EL, a surfactant which acted as the solubilizer agent in the marketed formulation Daktarin®. Another way is with the use of cyclodextrins. Though both means are capable of solubilizing adequate amounts of miconazole, both have disadvantages. Cremophor EL proved to have several side effects, including an anaphylactic shock-like reaction and cyclodextrins must be used in a very high concentration.

The previously raised issues and the fact that therapeutically effective parenteral formulations are needed for the treatment of systemic mycoses led us to the elaboration of the present work. The solvent system, which is a novel combination of well established exipients shows a synergetic solubilizing effect. The substantial solubilizing power of the solvent system, produced by the synergistic effect is able to solubilize therapeutically effective amounts of miconazole.

EXPERIMENTAL METHODS

The formulation steps of present work were divided into four steps: solubilizng miconazole, optimization of the solvents system, tests for sterilization and accelerated stability tests.
The solubility tests were conducted using pH adjusters, co-solvents, surfactants and their combinations. Solvent systems were prepared and an excess of miconazole was added to the solvents. After 30 minutes of shaking, the systems were let to reach equilibrium (72 hours). The excess active ingredient was filtered through a 0.45 μm pore size cellulose acetate membrane filter and an HPLC method was used for the quantitative determination of miconazole.

After reaching adequate solubility of miconazole, the composition of the ternary solvent system was optimized by reducing the amount of polysorbate and ethanol based on solubility and dilution tests. Dilution tests were performed using 5% dextrose and 0.9% sodium chloride infusions.

The stability and applicability of the solvent system was examined on autoclaving and on filtration. Stability of the solvent system was confirmed on autoclaving on 120°C for 20 minutes.

Static filter tests were conducted on 0.2 μm pore size cellulose acetate, cellulose nitrate and polyamide membrane filters. Integrity of the membrane was examined prior to and following testing and the ability of the membrane to bond miconazole was also examined.

Accelerated test of the solvent system was performed according to Guideline on stability testing: stability testing of existing active substances and related finished products.

RESULTS AND DISCUSSION

The solubility tests were systematically built up in order to achieve adequate solubility of miconazole. First aqueous solutions of the solubilizing excipients were examined, than binary and ternary solvent systems were tested. The solubilizing effect of one of the solvents systems from each group is shown in figure 1.

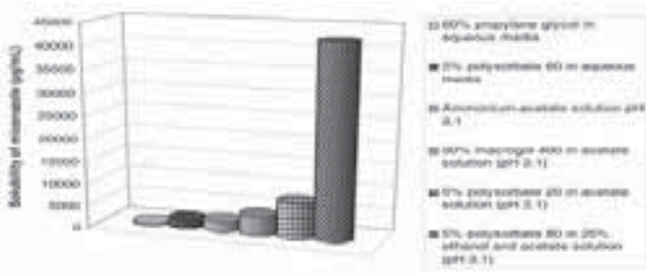

Figure 1: Solubilizing effect of various solvent systems

Our experiments led us to a non-excepted finding of an aqueous solvent system capable of dissolving hydrophobic pharmaceutically active agents in therapeutically effective amounts. The substantial solubilizing effect of the solvent system is based on the synergistic solubilizing effect of the applied ingredients. The solvent system comprises water, a pH-adjuster, a co-solvent and a surfactant all in quantities applicable in parenteral dosage forms. The solvent system is readily capable of solubilizing miconazole in water. More specifically a ternary solvent system comprising 5% polysorbate 80, 25% ethanol and 0.05M ammonium acetate (pH 3.1) is able of solubilizing more than 40 mg/mL of miconazole, which compared to its water solubility (~1μg/mL) is an increase of more than 40 000 solubility increase. Present novel combination of well established excipients combines their newly discovered advantageous effect on solubilizing and their already known insignificant toxicity.

Optimization of the solvent system was performed and resulted in a solvent system containing less excipients, but with a similarly substantial solubilizing ability.

The final composition was autoclaved and was found to be stable upon heat sterilization.

The tested membrane filters did not lose their integrity during the static filter tests, but the cellulose nitrate membrane filter absorbed substantial miconazole during the test. Therefore it was concluded that aside filtration with cellulose nitrate membrane filters, all of the tested methods of sterilization may be applied for the novel solvent system.

The ongoing stability tests prove the long term stability of the solvent system.

CONCLUSION

Based on the previous results it can be concluded that a novel solvent system was worked out, which showed a very substantial solubilizing power. After optimizing the composition of the solvent system it was shown that the solvent system is readily capable of being sterilized by both heat sterilization and filtration. The accelerated stability tests show long term stability of the solvent system. Based on these results a concentrate for infusion containing miconazole was formulated.

Reference

1. Strickley, R.G. Solubilizing excipients in oral and injectable formulations. Pharm. Res., 21(2), 201-230. (2004)
2. Yalkowsky, S.H. Solubility and Solubilization in Aqueous Media, New York. (1999)
3. Richardson, M.D., Jones, B.L. Therapeutic Guidelines in Systemic Fungal Infections, 3rd Ed., England. (2004)

PO083

Dissolution enhancement of quercetin using hydrophilic excipients

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INTRODUCTION

Quercetin is a well known flavonoid highly distributed in food, particularly in vegetables and fruits. It has been extensively investigated due to its promising pharmacological activities including antioxidant, hepatoprotective, antiinflammatory and antitumor effects. Nevertheless, its use in clinical field is very limited due to its low solubility and slow dissolution rate in water determining poor drug bioavailability after oral administration. In this work the possible increase of quercetin dissolution rate has been investigated. To this goal solid binary mixtures between quercetin and two hydrophilic excipients (β-cyclodextrin, β-CD, and polyethyleneglycol 4000, PEG4000) have been prepared by kneading, freeze-drying solvent evaporation and hot-melting methods. Furthermore, the physical state of the drug has been investigated by differential scanning calorimetry (DSC) and powder X-ray diffractometry (PXRD).

EXPERIMENTAL METHODS

Sample preparation

Quercetin, β-CD and PEG4000 were previously sieved and the granulometric fraction < 250 μm was used to obtain solid binary mixtures.

Physical mixtures were prepared by simple mixing different amounts of Quercetin with fixed amount of each excipient to obtain samples in which quercetin concentration varied between 10 and 90% w/w.

Kneaded mixtures were prepared adding small amounts of water to each excipient placed in different mortars and mixing with a pestle until a homogeneous paste was obtained. Then, the required amount of quercetin powder was slowly added to the paste kneading the mixture for 45 minutes. The resulting samples were dried in an oven at 45 °C for 48 hours and the solids were ground and sieved through a 250 μm sieve. Also these samples contained quercetin concentration ranging between 10-90% w/w.

Freeze dried mixtures were prepared dissolving the excipient in water and adding a suitable amount of quercetin to obtain a 1:1 molar ratio mixture. The suspensions were left 48 hours under stirring at room temperature then were filtered through 0.22 μm membrane filter and the clear solutions were congealed and freeze-dried. The obtained solids were ground, sieved through a 250 μm sieve and mantained at 50 °C in an oven for 48 hrs. Binary mixtures between quercetin and PEG4000 (quercetin concentration ranging between 10 and 90 % w/w) were also obtained by solvent evaporation, in which quercetin and PEG4000 were dissolved in ethanol and solvent was evaporate in a rotary evaporator, and melting methods in which suitable amounts of quercetin were added to fixed amount of melted PEG4000. The mixtures were then cooled and the ground solids were sieved through a 250 μm sieve.

Differential scanning calorimetry

Thermal analyses were performed using a Mettler TA 4000 apparatus equipped with a DSC 20 measuring cell. Samples of drug alone, excipients and all the binary mixtures, containing 1.5 mg of quercetin, were accurately weighed in aluminium pans wich were sealed and heated at a scanning rate of 10 °C/min from 30 to 330 °C using an empty aluminium pan as a reference. Temperature and heat flow calibrations were carried out using indium as a standard.

Powder X-ray diffractometry

The crystallinity of quercetin in the drug powder and all binary mixtures was determined at room temperature using a Siemens powder diffractometer with a Cu-K radiation (λ= 1.5418 Å), a current of 20 mA and a voltage of 40 kV. Each sample wase analysed in the 2 angle range between 3 and 40 degrees with a scan step size of 0.05.

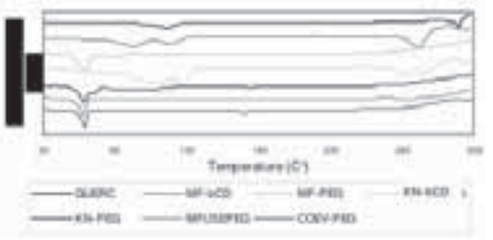
Dissolution studies

The USP XXIV rotating paddle apparatus was used to obtain dissolution profiles of drug alone and drug-excipient binary mixtures. Dissolution medium (900 ml of water) was mantained at 37 ± 0.5 °C and stirred at 100 rpm. Powdered samples (with granulometric dimension < 250 m), containing a suitable amount of quercetin to mantain sink conditions (C < 0.2Cs), were added over the surface of the dissolution medium. The aqueos solution was continuosly filtered and pumped to a flow cell in a spectrophotometer and absorbance values were recorded at 256 nm. Dissolution tests were carried out at least three times for each sample and the mean value and standard deviation were calculated.

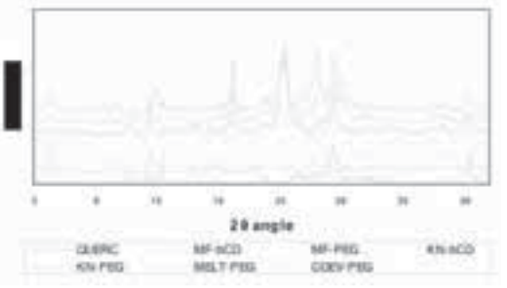
RESULTS AND DISCUSSION

DSC thermograms are reported in the following figure.

Quercetin showed an endothermic peak at 320 °C due to drug melting and an endothermal event between 95-110 °C due to dehydration of the crystalline drug molecules. Both binary mixtures with β-CD showed a broad endothermal event ranging from 280-310 °C attributable to drug melting. Peaks broadening and lower drug melting temperatures suggested great interactions between drug and CD. In all PEG binary mixtures drug melting peak totally disappeared. This phenomenon was probably due to the solubilization of the drug in the melted PEG during DSC experiments.

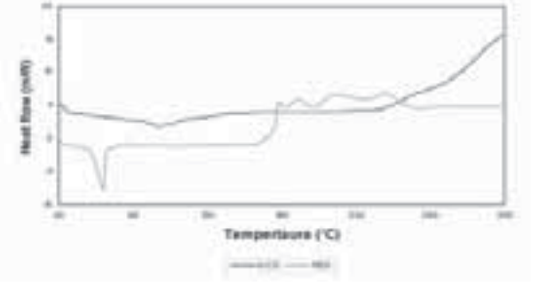


X-Ray diffractogram, reported in the following figure, confirmed data obtained from thermal analyses.



All binary mixtures shoved several peaks attributable to the crystalline state of the drug even if the peak intensities were decreased indicating a partial amorphization of the drug. Drug cristallinity in PEG binary systems confirmed that the disappearence of drug melting event in DSC analysis was due to drug dissolution in melted PEG

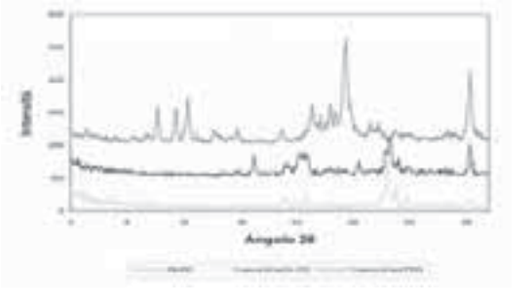
In the following figure dsc curves of freeze-dried binary mistures have been reported.

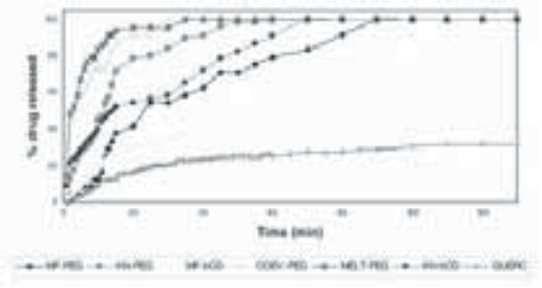


No endothermal event attributable to the drug melting was detected suggesting totally amorphized state of the drug in these systems. Exothermal events seems suggest degradation of the excipient during heating. As observed in x-ray diffraction analysis characteristic diffraction peaks of crystalline drug disappeared confirming the amorphous state of the drug. Only some peaks of crystalline PEG and cyclodextrins were detected.

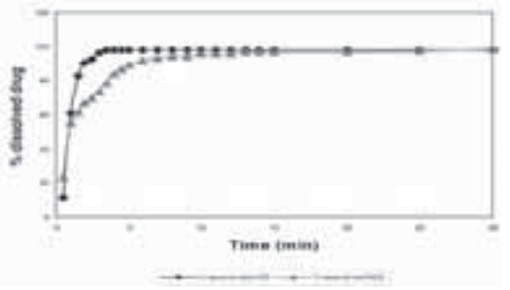
Dissolution rate measurements, reported in the following figure, showed a great increase of drug dissolution for all prepared binary mixtures

compared to the drug alone. Several explanation can be proposed for this effect: reduction of crystalline particle size, improved wettability, solubilization effect of the carrier, partial amorphization of the drug and the combination of these mentioned mechanisms.





Best drug dissolution rates were obtained with freeze dried systems in which the drug was present in amorphous form. For β-CD product the formation of an inclusion complex was also possible.



CONCLUSION

Both cyclodextrin and PEG4000 are able to increase the dissolution rate of quercetin through several possible mechanisms. Best results are obtained by freeze dried binary mixtures in which the drug was in amorphous state.

PO084

Alendronic Acid with Poloxamers formulations

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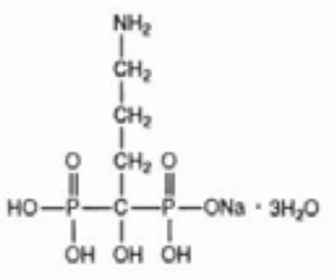
INTRODUCTION

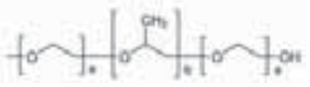
Biphosphonate, a salt, ester or anion of a dimer of phosphonic acid (diphosphonic acid) inhibits bone resorption as a sodium salt. The monophosphonates are not active. Biphosphonates are used in disorders such as osteoporosis, metastatic disease and Paget s disease. Biphosphonates are analogues of endogenous pyrophosphate structurally, they are characterized by a P-C-P bond, the linking oxygen is replaced with a carbon, which is resistant to enzymatic and chemical hydrolysis. Side chains are responsible for chemical-physical properties, mechanism of resorption and pharmacokinetics. Biphosphonates bind strongly to hydroxyapatite crystals at the sites of increased bone turnover preferentially and inhibit the formation, aggregation and dissolution of the crystals.

Alendronate sodium is a second generation bisphosphonate It directly inhibits multiple steps in the pathway from mevalonate to cholesterol and isoprenoid lipids, such as geranylgeranyl diphosphate that are required for the prenylation of various proteins that are important for osteoclsat function. Chemically, it is described as (4-amino-1-Hydroxybutylidene) bisphosphonic acid monosodium salt trihydrate.



It is soluble in water. It is in the Class III of the Biopharmaceutical Classification System with high solubility and low permeability. It is absorbed very poorly from the intestine. [1,5]

Poloxamers are block-copolymers consisting of Polyoxyethylene and Polyoxypropylene units.



The chracter of each poloxamer in terms of molecular weight, appearance, hydrophilicity hydrophobicity, solubility is determined by the chain length of the Polyoxyethylene and Polyoxypropylene units. [2,3,4]

Uses of poloxamers	Conc. (%)
Fat emulsifier	0.3
Flavor solubilizer	0.3
Fluorocarbon emulsifier	2.5
Gelling agent	15–50
Spreading agent	1
Stabilizing agent	1–5
Suppository base	4–6 or 90
Tablet coating	10
Tablet excipient	5–10
Wetting agent	0.01–5

OBJECTIVE

Although Alendronate sodium trihydrate is an active substance soluble in water, we wanted to achieve better in vitro solubility compared to our referent formulation which is still within the compendial requirements. According to USP 30-NF 25 Monograph for Alendronic acid tablets not less than 80% (Q) of the labeled amount of alendronic acid ($C_4H_{13}NO_7P_2$) has to be dissolved in water in 15 minutes. We have used micronised grades of poloxamers to achieve better solubility in vitro and probably bioavailability enhancement also.

MATERIALS

Alendronate sodium trihydrate "AUROBINDO", India; Poloxamer 188 and 407 micronised (Lutrol micro 68 and 127 "BASF", Germany); Microcrystalline cellulose PH12 (Vivapur 12 "JRS", Germany); Dibasic Calcium Phosphate anhydrous (Emcompress"JRS",Germany); Copovidone (Kollidon VA64 "BASF", Germany); Crosspovidone (Polyplasdone XL-10 "ISP", Germany); Sillica colloidal, anhydrous (Aerosil 200 "Evonik", Germany); Talc (Talk "Merck",Germany; Sodium stearyl fumarate (PRUV "JRS", Germany).

METHODS

MANUFACTURE OF TABLETS:

Direct Compression:

The active substance together with the poloxamers was manually sieved through 0.8 mm several times. Then they were mixed in Erweka Double Cone Mixer Type DKM for 30 min, 125 rpm. Then all the other excipients except lubricant were added, manually sieved through 0.8 mm sieve, and mixed for 15 min.

Lubricant was added, mixed for 5 min. and tablets were produced by direct compression on Erweka Type EKO single punch tablet press, with constant compression force. Physical parameters of the tablets were recorded and evaluated.

% in the formulation of Alendronat tbl. 10 mg	Formulation A	Formulation B	Formulation C	Formulation D	Formulation Ref.
Alendronate Na x $3H_2O$	7.25	7.25	7.25	7.25	7.25
Lutrol® micro	68	3	5		
Lutrol® micro 127			3	5	
Other ingredients	87.25	85.25	87.25	85.25	90.25
Polyplasdone XL-10	0.5	0.5	0.5	0.5	0.5
PRUV®	2	2	2	2	2

TEST METHODS:

Hardness testing: Hardness tester TBH 100, "Erweka"

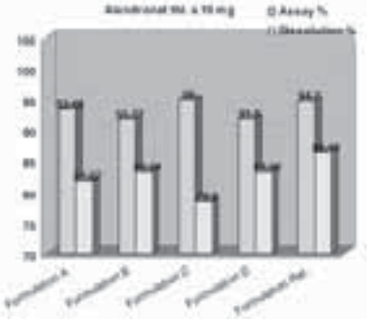
Disintegration testing: ZT 70 Series "Erweka"

Assay: As specified in USP30-NF25, Alendronic acid tablets Monograph; "Perkin Elmer" Lambda 45 UV/VIS spectrophotometer

Dissolution testing: As specified in USP30-NF25, Alendronic acid tablets Monograph: water, 900 ml, Apparatus 2, 50 rpm, 15 min. Tolerances: not less than 80% (Q) of the labeled amount of alendronic acid ($C_4H_{13}NO_7P_2$) has to be dissolved in 15 minutes. Tablet dissolution tester "Sotax AT 7 Smart with "Perkin Elmer" Lambda 45 UV/VIS spectrophotometer

RESULTS AND DISCUSSION

Formulation	A	B	C	D	Ref.
Tablet weight (mg)	180				
Tablet shape	8 mm, round, biconvex				
Tablet Hardness (N)	69.5	72.0	69.0	67.0	68.4
Disintegration time	1 min. 45 sec.	5 min.	2 min.	8 min.	16 sec.
Assay (%)	93.48	91.77	95.00	91.90	94.70
Dissolution (%)	81.87	83.38	78.60	83.36	86.46



CONCLUSIONS:

- Poloxamers 188 and 407 micronised, used in the concentrations of 3 and 5% in the formulation did not improve the solubility of Alendronic acid, tested according to USP30-NF25.

- According to literaure data [3], higher levels of poloxamer create a greater binding effect which has an impact on dissolution. But there is data where the actives and Poloxamers are used in 1:1, 1:3, 1:5, 1:10 ratio i.e. more than 3% and 5% in the formulation [3]. According to other literature data, poloxamers should be used as wetting agents in concentrations of 0.01–5%[4]. That is why more formulations with different (than 3% and 5%) concentrations of poloxamers have to be tried.
- The direct compression as a manufacturing method did not give dissolution enhancement. Other manufacturing methods have to be tried.

References:
1. Hardman, Limbird, Gilman "The Pharmacological basis of therapeutics" (2001)
2. "Encyclopedia of Pharmaceutical Technology", (2002;2007)
3. Dr. Michael Black "Characterization of Newly Developed Micronized Poloxamers for Poorly Soluble Drugs", lecture on Pharma Solutions meeting, Budapest (2007)
4. R.C.Rowe, P.J.Sheskey, S.C.Owen "Handbook of Pharmaceutical Excipients" (2005)
5. USP 30/NF 25 (2006)

PO085

Self-emulsifying drug delivery systems containing pindolol

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INTRODUCTION

The self-emulsifying drug delivery systems (SEDDS), whereby drugs are solubilised in an oil-surfactant mix that emulsifies on contact with water, represent a promising approach for enhancing oral bioavailability of hydrophobic drugs,with adequate oil solubility.

Depending on the composition, the SEDDS transform on contact with the release medium and under moderate stirring into emulsions, liquid crystals or microemulsions. The efficiency of SEDDS depends on two main factors: the uniform fine particle size of oil droplets on contact to aqueous media and the polarity of the resulting oil droplets. Both properties control the rate of the drug release from the oil to the external phase.

One of the most important properties of a drug delivery system is the release profile.

Washington and Evans [1] described a method widely applicable to the release study from colloidal systems of drugs with weak acidic or basic properties. This method highlights rapid release of drugs from submicron emulsions and microemulsions [2] by monitoring the change in pH of an acceptor phase as the release system is diluted into it.

In this study the release of pindolol from SEDDS is studied by monitoring the change in pH of the release medium. The influence of an ion pair formation on the drug release was also examined.

EXPERIMENTAL METHODS

• SEDDS formulation and characterization

After preliminary experiments on the drug solubility, glycerol 1-monooleate (GMO) was chosen as oil in SEDDS preparation. Self-emulsifying systems based on Epikuron 200 (soybean lecithin, E200), Oramix NS 10 (decyl polyglucoside, O10) or Brij 30 (Laureth-4, B30) as surfactants and propylene glycol (PG) as cosolvent were prepared.

The self-emulsifying capacity of SEDDS was determined by the measurement of the transmittance (800 nm) over time of the emulsions obtained after deposition of an aliquot of SEDDS on the release medium.

The size of the emulsion droplets obtained after SEDDS dilution was determined by LLS.

Drug-loaded SEDDS containing 0.7% pindolol in the presence or in the absence of decyl phosphate ($C_{10}P$) as counter ion were prepared.

• Pindolol release studies

Drug release was determined according to [1], by determining the pH change over time after addition of 0.5 ml of SEDDS into 100 ml of HCl at pH=3.5 in a 150-ml container fluxed with N_2. pH was measured until no further change occurred (pH_∞). Measurements were performed under magnetic stirring at 25°C in triplicate.

Since 1 mol of released drug neutralised 1 mol of hydrogen ions, the fraction of the released solute is given by:

$$\frac{M_t}{M_0} = \frac{10^{-pH_0} - 10^{-pH_t}}{10^{-pH_0} - 10^{-pH_\infty}}$$

M_t is the amount of released drug at time t=10 s.
The release of pindolol as ion pair was determined at 30 s by HPLC owing to the interference of counter ion on pH measurement.

RESULTS AND DISCUSSION

The drug solubility in GMO was higher than in other oils (Table 1).

Table 1: Solubility of Pindolol in oils

Oil phase	Solubility (mg/ml)
GMO	11.5
Miglyol 810	0.9
Miglyol 818	0.6
Corn oil	1.2

In table 2 the compositions of GMO-SEDDS are reported.

Table 2: SEDDS compositions (% w/w).

	1A	1B	1C	2A	2B	2C	2D	3A	3B	3C
GMO	10	10	10	10	10	10	10	10	10	10
E200	50	40	30	3	5	25	45	-	-	-
O10	-	-	-	47	45	25	5	-	-	-
B30	-	-	-	-	-	-	-	50	40	30
PG	40	50	60	40	40	40	40	40	50	60

In Table 3 the size of the emulsion droplets obtained after SEDDS dilution in the release medium at t=0 and t=24 h is reported.

Table 3: Droplet size (nm) SD of the emulsions after SEDDS dilution.

Formulation	Droplet size (nm) ± SD t=0(nm)	Droplet size ± SD t=24h
1A	508.7 ± 12.2	476.3 ± 15.7
1B	500.2 ± 10.3	483.4 ± 23.4
1C	498.7 ± 16.2	400.3 ± 12.5
2A	211.3 ± 12.6	235.7 ± 13.1
2B	214.8 ± 11.5	204.4 ± 15.1
2C	335.4 ± 12.1	324.2 ± 13.5
2D	501.3 ± 13.5	467.3 ± 10.2
3A	405.3 ± 19.8	398.7 ± 12.6
3B	308.8 ± 12.0	270.4 ± 13.2
3C	304.1 ± 12.8	281.2 ± 11.3

The transmittance variation of the emulsions normalised by the transmittance values at 24 h (T/T_{24h}) vs time is reported in figure 1. The efficiency of SEDDS is reduced in the systems with high lecithin percentage.

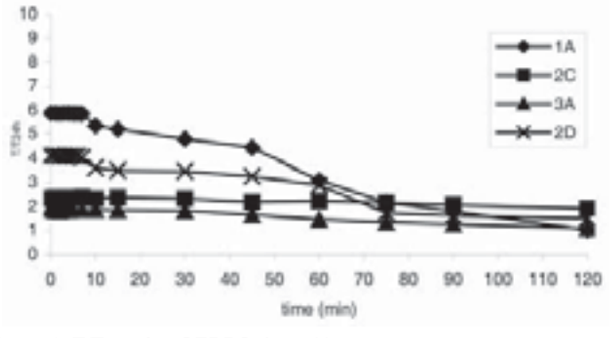

Figure 1: T/T_{24h} after SEDDS deposition.

The low release values obtained from formulations 1 may be due to the poor emulsification capacity (Figure 1) and to the large droplet size. The formulations 2 have a small droplet size and a high self-emulsification capacity leading to a fast drug release.

The increased Oramix NS10/Epikuron 200 weight ratio used in the formulations 2 could lead to an increased hydrofilicity of the interface, determining an increased drug release.

In Table 4 the fraction of drug released (M_t/M_0 x100) from SEDDS, is reported.

Table 4: Percentage of drug released at t=10 s.

Formulation	M_t/M_0 x100
1A	8
1B	37
1C	49
2A	83
2B	81
2C	75
2D	25
3A	62
3B	57
3C	54

The low values obtained for formulations 3 as compared with formulation 2 A, B and C, may be due to the different characteristics of the interfacial layer of the systems and to the large droplet size of the emulsions despite the ease of self-emulsification capacity.

In table 5 the fractions of pindolol released (M_t/M_0 x100) at 30 s with and without counter ion, from formulation 2, are reported.

Table 5: Percentage of drug released at t=30 s as pindolol or pindolol/$C_{10}P$.

Formulation	M_t/M_0 x100 pindolol	M_t/M_0 x100 pindolol/$C_{10}P$
2A	100	64
2B	100	87
2C	84	50
2D	32	13

The release of pindolol was reduced for all the SEDDS in the presence of ion pair.

CONCLUSION

SEDDS with good self-emulsification capacity were obtained using Brij 30 or a mixture of Oramix NS10/Epikuron 200 with low lecithin percentage. The drug releases observed from SEDDS are not suitable to obtain a prolonged drug delivery system. The effect of ion pair could significantly help to reduce the drug release from SEDDS.

References

1. Washington C., Evans K. Release rate measurements of model hyrophobic solutes from submicron triglyceride emulsions. J. Control. Release (1995) 33: 67-78.
2. Trotta M. Influence of phase transformation on indomethacin release from microemulsions. J. Control. Release (1999) 60: 399-405.

PO086

SNEDDS pellets produced by wet granulation process to increase piroxicam dissolution rate

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INTRODUCTION

The absorption of poorly water-soluble drugs can be limited by the dissolution rate and the extent to which the drug dissolves. The thermodynamic principles controlling drug solubilisation indicate that an increase of solubility is obtained by reducing intermolecular forces in the solid state and enhancing solute-state interactions in the bulk solution. Strategies to improve drug solubility by the alteration of solid-state properties include identification of advantageous polymorphs, hydrates or salts [1]. The rate of dissolution can be enhanced by reducing particle size or manufacturing solid dispersion formulations among which SNEDDS (self-nanoemulsifying drug delivery system).

SNEDDS are self-emulsifying systems containing a surfactant, a co-surfactant an a lipid phase that after oral administration spontaneously form *in-situ* a nanoemulsion containing the drug in molecular dispersion. These systems are liquid or semi-solid formulations that care commonly admistered in soft gelatin capsules which have some disadvantages such as high production costs and difficulties in administration [2].

The goal of the present study was to apply the wet granulation process in high shear mixer to develop SNEDDS pellets to increase oral biodisponibility of poorly water soluble drugs by increasing dissolution rate.

EXPERIMENTAL METHODS

• Pseudo-ternary phase diagrams

Solubility of piroxicam (model drug) in various oils, surfactants and co-surfactants was determined. Pseudo-ternary phase diagrams of oil, surfactant, cosurfactant and water were developed using the water titration method [1]. Different phase diagrams at the specific ratios of surfactant/cosurfactant, 1:1, 1:2, 2:1 (*w/w*) respectively were prepared.
After the identification of the nanoemulsion regions in the phase diagrams, the nanoemulsion formulations were selected at specific component ratios.

• SNEDDS pellets production

SNEDDS pellets were prepared using the following materials: microcrystalline cellulose (Flowcel 101®, Gujarat Microwax, pvt LTD, India), monohydrate lactose (Granulac 200®, Meggle, Germany), propylene glycol monolaurate (Lauroglycol 90®, Gattefossè, France) as oil phase, diethylene glycol monoethyl ether (Transcutol HP®, Gattefossè, France) as co-surfactant, Polyoxyl 35 castor oil (Cremophor EL®, BASF, Germany) as surfactant, Piroxicam (FIS, Italy) and fresh demineralised water. In 1 l laboratory scale high shear mixer (Rotolab®, Zanchetta, Italy), the granulation procedure and the process variables (impeller speed, massing time, percentage of liquid phase) were fixed on the basis of the preliminary trials. The emulsions were prepared mixing oil, surfactant, cosurfactant, piroxicam and water in variable ratios by magnetic stirrer. After preliminary mixing of the powders (lactose and microcrystalline cellulose) at 120 rpm for 10 min, the nanoemulsion was added dropwise while stirring at 120 rpm to obtain a uniform distribution. Massing time was fixed at 5 min and the impeller speed at 1200 rpm. At the end of the granulation process the pellets were dried at 40°C until they reached a constant weight and then stored in sealed bags before characterisation. After the sieve analysis, dissolution tests and laser light scattering measures were performed. Dissolution tests and laser light scattering measurements were realised in simulated intestinal fluid (pH 7.4).

RESULTS AND DISCUSSION

Lauroglycol 90® and Transcutol HP® were selected as oil and cosurfactant for the SNEDDS formulations due to their higher solubilities for Piroxicam. Pseudo-ternary phase diagrams (figure 1) were constructed to identify the self-emulsifying regions in order to optimize the concentrations of oil and corsurfactant. The compositions of the two formulations selected are reported in table 1. Both formulations were used to produced SNEDDS pellets which were characterised by sieve analysis. The dissolution tests and laser light scattering measurements were performed on the 400 μm pellets fraction.

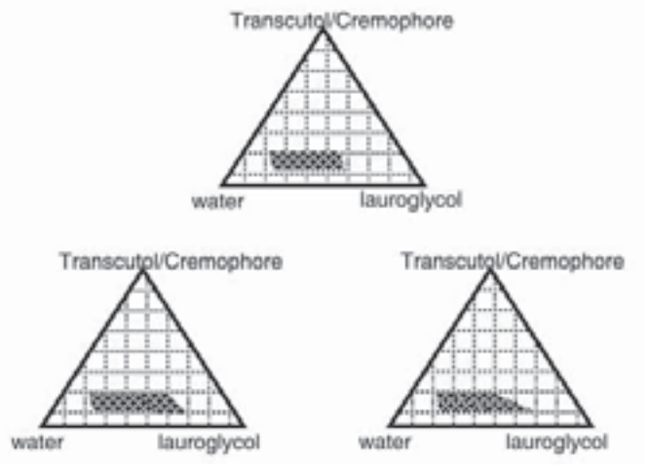

Figure 1: Pseudo-ternary phase diagrams indicating the efficient self-emulsification region (a-transcutol/cremophore ratio 1:1 (w/w); b-transcutol/cremophore ratio 1:2 (w/w); c-transcutol/cremphore ratio 2:1 (w/w)).

Laser light scattering measurements demonstrated that pellets produce spontaneously SNEDDS when put in simulated intestinal fluid. SNEDDS formulations produced emulsion with a mean particle size of 500 nm.

The dissolution profiles are reported in figure 2. The profiles demostrate the SNEDDS ability to increase the drug dissolution rate.

Table 1: Compositions of the SNEDDS formulations containing piroxicam.

Formulation	A	B
Water (%)	70	50
Lauroglycol 90 (%)	20	30
Transcutol HP (%)	6,7	13,3
Cremophore EL (%)	3,3	6,7

CONCLUSIONS

This study demonstrates the ability to produce SNEEDS pellets by a wet granulation process in high shear mixer and to increase the dissolution rate of a poorly water-soluble drug like piroxicam.

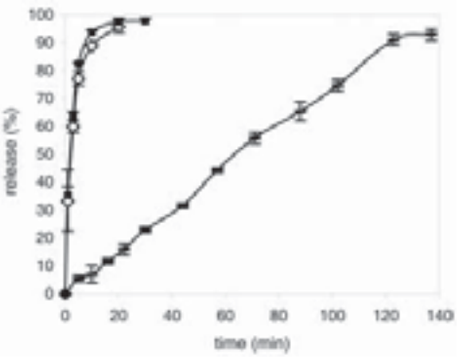

Figure 2: Dissolution profiles of: ◇ SNEDDS pellets A; ■ SNEDDS pellets B; ▬ piroxicam

References

1. Pouton C.W. Formulation of self emulsifying drug delivery systems. Advanced Drug Delivery Reviews 25: 47 - 58 (1997).
2. Bok Ki Kang et al. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. International Journal of Pharmaceutics 274: 65-73 (2004).

PO087

Physical Characterization and Dissolution Behavior of Simvastatin – Gelucire 44/14 Solid Dispersion

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Key words: simvastatin, In-vitro dissolution, solid dispersion and physical characterization

INTRODUCTION

Simvastatin, a HMG Coenzyme reductase inhibitor mainly used as a hypolipidemic agent. One of the major problems with this drug is, it poorly absorbed from the gastrointestinal (GI) tract because it is practically insoluble in water (0.003mg/ml) and according to the BCS classification simvastatin comes under class II (Low Solubility and High Permeability) thus, dissolution is a rate-limiting step for its absorption. Therefore, it is very important to introduce effective methods to enhance the dissolution rate of the drug, substantially leading to its bioavailability.

In the light of above fact, the present study has been undertaken with following objectives.

1. To improve the dissolution of simvastatin by preparing solid dispersion Gelucire 44/14
2. The Most promising batch was compare with simvastatin plain tablet and market tablet.

EXPERIMENTAL WORK

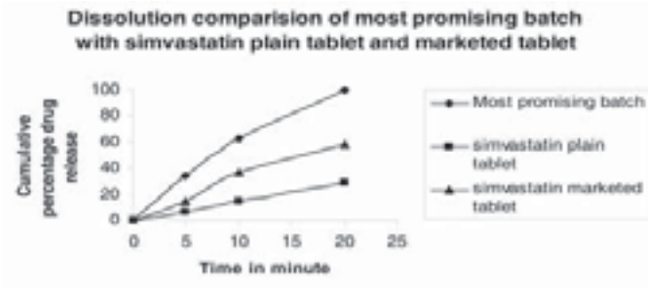
Dissolution enhancement by preparing Solid dispersion: Gelucire 44/14 was used as carrier for preparing solid dispersion. Solid dispersions were prepared using fusion method in the ratio from 1:1 to 1:3 (Drug to Polymer).

In vitro dissolution of most promising batch was compared with simvastatin plain tablet and simvastatin market tablet. In vitro Dissolution performed in Phosphate buffer pH 7.0 containing 0.1% SLS at 50 RPM, 37 ± 0.5^0C. Most promising batch was characterized by XRD, DSC, SEM and FT-IR study.

RESULT AND DISCUSSION

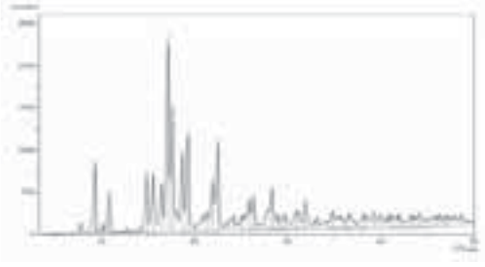
Tablet containing solid dispersion with gelucire 44/14 in the ratio of 1:3 (drug: polymer) exhibited fastest drug release (100 % drug release in 20 minute) that's why it considered as a most promising batch. The most promising batch was compare with simvastatin plain tablet and simvastatin market tablet for f1 (dissimilarity factor) and f2 (similarity factor) statistics. Value indicated there is a significant difference in dissolution

profile of most promising batch with simvastatin plain tablet and simvastatin market tablet.

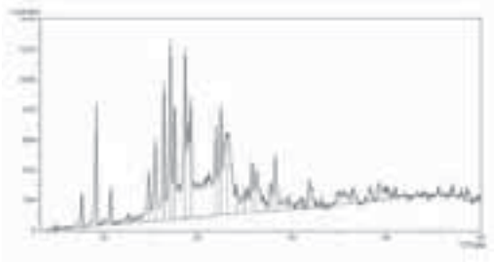


Physical Characterization:

(1) XRD Spectra

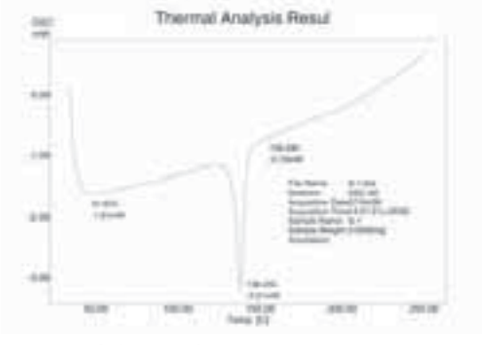


XRD Spectra of simvastatin

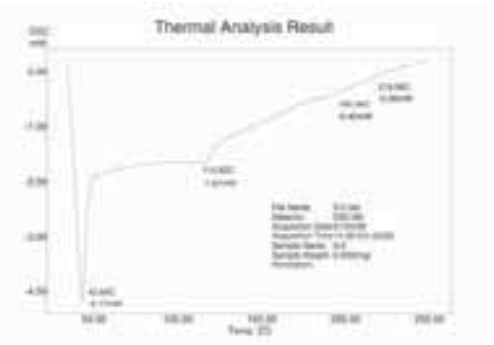


XRD Spectra of most promising batch

Result of XRD Spectra: Comparison of XRD spectra indicated that reduction of peak height (50 %) and presence of background peak showed in most promising batch. It indicated crystallanity of simvastatin reduce which responsible for dissolution enhancement



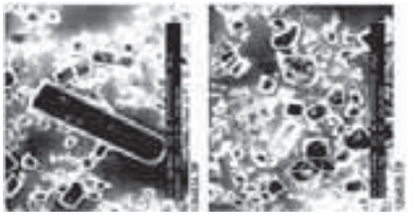
DSC thermogram of simvastatin



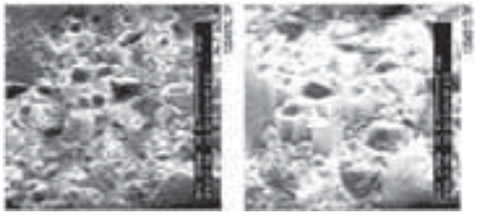
DSC thermogram of most promising batch

Result of DSC thermogram: Simvastatin showed a melting endotherm at 138.23^{0}C. In thermogram of solid dispersion with Gelucire 44/14, a characteristics sharp endothermic peak of simvastatin in the range of 130^{0}C - 139^{0}C was absent. It indicated solubilisation of simvastatin in molted carrier or partial amorphization of simvastatin**.**

(3) SEM study



SEM of simvastatin



SEM of simvastatin-gelucire 44/14 solid dispersion

SEM revealed effective formation of solid dispersion of simvastatin with gelucire 44/14 because well defined difference in morphology of simvastatin and solid dispersion.

(4) FT-IR study

Overlay FTIR spectra of simvastatin and most promising batch.

Result of FT-IR Spectra: The main simvastatin absorption band at 3546, 1718, 1459, 1267, 870 cm^{-1} (attributed to the Free O-H stretching, Lactone C= O stretch, Methylene C-H symmetric bend, lactone C-O-C stretch, Trisubstituted olefienic wag respectively) were remain unchanged in prepared solid dispersion, it indicated there is no chemical interaction between simvastatin and Gelucire 44/14.

CONCLUSION

Solid dispersion is promising formulation strategy for dissolution enhancement of simvastatin. Prepared solid dispersion gave 3.49 times and 1.72 times faster dissolution as compared to simvastatin plain tablet & market tablet respectively.

PO1088

The influence of atomizing pressure and feeding rate on the particles size in a spray congealing process

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INTRODUCTION

Spray-congealing (also spray-chilling, spray-cooling) is one of many methods that can be used to produce microparticles and is gaining considerable attention, especially from the safety and rapidity point of view (1). Microparticles are formed by atomizing the molten excipient into droplets which subsequently solidify upon cooling. This is advantageous because the use of aqueous phase or organic solvents can be avoided, drug release can be modified, taste can be masked and drug can be protected from the influence of environment (2,3). Spray-congealing can be used either to prolong the drug release or to increase the drug release – depending on the molten material used (2). The droplet size and consequent microparticles size can be controlled by the atomizing conditions (3). There are several types of atomizers, however dual-fluid nozzle allows the best control of particle size because the atomizing pressure and liquid feeding rate can be controlled independently (2).
The aim of this study was to successfully prepare spherical microparticles. We also studied the effect of feeding rate and atomizing pressure on microparticles size distribution by observing the amount of useful fraction of the product.

EXPERIMENTAL METHODS

Büchi Mini Spray Dryer B-290 (Büchi, Switzerland) was used for microparticles preparation. Microparticles were prepared from Gelucire 50/13 (stearyl macrogolglycerides or polyoxylglycerides, solid pastilles, HLB = 13, Gattefossé, France). The material was melted and heated to 70 °C, then it was fed to the atomizing nozzle using peristaltic pump via heated tubing to prevent solidification. Heated dual-fluid nozzle with 0.7 mm nozzle tip and 1.5 mm nozzle cap was used to spray Gelucire into the cooling chamber.

Total yield was calculated from the final mass of product. The product was then sieved through 125 μm and 500 μm sieve (Retsch, Germany) – the fraction between the sieves was designated as *useful fraction*. Microparticles were also examined by scanning electron microscope (Zeiss SUPRA35 VP, Germany).

RESULTS AND DISCUSSION

Effect of feeding rate

The effect of feeding rate on particle size was studied in a series of four batches, produced under the same conditions except for varying feeding rate.

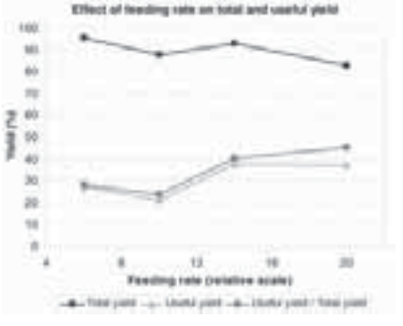

Figure 1: Effect of feeding rate on total and useful yield at atomizing pressure of 1440 mbar.

Total yield was over 82% for all batches. There is a clear trend of increasing useful yield with the increased feeding rate. All other parameters being equal, a faster feeding rate increases the droplet size during atomization (4). In a spray-congealing process this is clearly seen as an increase of particle size which is measured as a useful fraction. As the feeding rate further increases, total yield starts to decrease slightly due to increased droplet sticking to the chamber wall. This means that the feeding rate must be optimized and a compromise between the particle size and total process yield must be made.

Effect of atomizing pressure

The effect of atomization pressure was studied in a series of five batches, produced under the same conditions except for varying atomizing pressure. All batches have total yield over 80%. A higher atom-

ization pressure increases the shear stress on the droplet when the molten material is exiting the nozzle. This leads to smaller droplet size (4), which is shown by a distinct decrease in useful yield. When atomization pressure is decreased below 1100 mbar, the droplets are becoming too big to solidify before hitting the wall of the cooling chamber. This results in a decrease of total yield (which lowers useful yield as well) and in formation of distinct layer of material at the top of the cooling chamber.

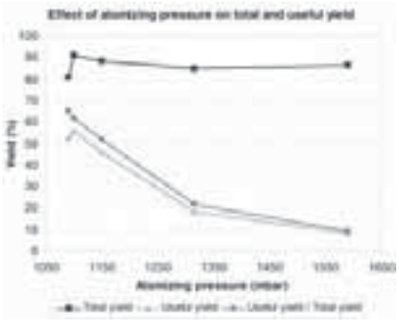

Figure 2: Effect of atomizing pressure on total and useful yield at relative feeding rate of 6.

When we compare the influence of feeding rate and atomizing pressure, we can influence particle size more by changing atomizing pressure rather than changing liquid feeding rate – this is in agreement with literature data (4).

SEM analysis

Microparticles were successfully prepared by spray congealing. The process yield was optimized to over 90%. Useful fraction (particle sizes from 125 to 500 μm) represents approximately 60% of the product mass. The useful fraction and bottom fraction (particles smaller than 125 μm) were analyzed using SEM in order to evaluate the particle shape and size after performing sieve analysis.

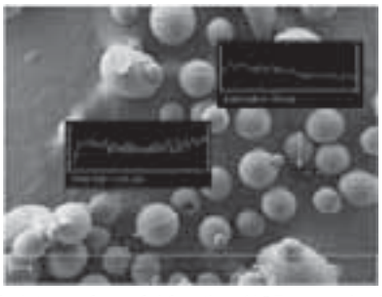

Figure 3: SEM picture of useful fraction (125 μm < d < 500 μm) with diameter size measurements.

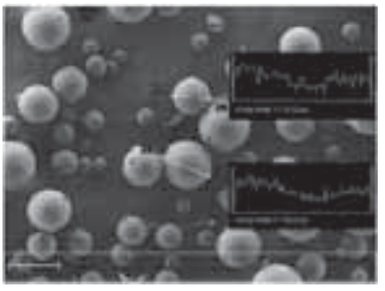

Figure 4: SEM picture of bottom fraction (d < 125 μm) with diameter size measurements.

From the SEM analysis we can conclude that the product is mostly in the form of primary particles which are almost completely spherical and have a relatively smooth surface. SEM particle size measurements of useful and bottom fraction are in accordance to the sieve mesh sizes used during sieve analysis.

Conclusion

Microparticles from Gelucire 50/13 were successfully prepared with relatively high process yields (from 80 to 95%). Scanning electron microscopy shows that the product consists mainly of primary particles of ideal spherical shape with smooth surface.

Particle size of microspheres depends on many parameters including feeding rate of molten material and atomizing pressure. Higher feeding rates increase the particle size, however, total yield shows a slightly decreasing trend at these conditions – therefore a moderate feeding rate is suggested for optimal results. Lower nozzle pressure decreases the shear forces on the droplet resulting in particle size increase. When the droplets of molten material are too big, the particles will not solidify before hitting the wall of cooling chamber what decreases total process yield. Optimization of atomizing pressure again requires a compromise between particle size and total process yield. A greater change in particle size can be obtained by varying atomizing pressure at a constant feeding rate rather than by changing feeding rates while maintaining constant atomizing pressure.

References

1. B. Albertini, N. Passerini, et. al., Eur. J. Pharm. Biopharm. 69:348-357 (2008)
2. R. Turton, X. X. Cheng, Cooling Processes and Congealing in J. Swarbrick, Encyclopedia of Pharm. Tech. 3rd Ed., 761-773, Informa Healthcare, USA (2007)
3. A. Maschke, C Becker, et. al., Eur. J. Pharm. Biopharm. 65:175-187 (2007)
4. L.S.C. Wan, P.W.S. Heng, C.V. Liew, Int J Pharm 118:213-219 (1995)

PO089

Comparison of models determined by impeller torque and acoustic signal analysis on process parameters effecting pelletizing end-point

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INTRODUCTION

Pelletizing applying a high-shear granulator is a multivariate process. Controlling process variables various pharmaceutical and biopharmaceutical properties of pellets could be modified. Crucial parameter of pelletizing is the optimal end-point (Ep). At this point size and size distribution of pellets, roundness and several other variables reach optimal value. There are several possibilities to determine the end-point of pelletizing. Methods collect datas of impeller torque, power consumption or rheological properties in order to find the end-point. Alternative investigations were already tested to get more information on the pelletizing and to find the end-point as well. Techniques of measuring sound, vibration, rapid image processing, NIR moisture sensor, Focused Beam Reflectance Measurement (FBMR) are all novel methods to improve the determination of end-point. Our experiments monitored with digital acoustic analysis seem to be adequate to expand the evaluating methods of high-shear pelletizing. Acoustic analysis was already tested by a few investigators. Whitaker at al monitored sound signals from the bottom of the granulation bowl. Lack of this method was the uncertain determination of the end-point. Briens et al measured sound signals at various locations of the granulation bowl thus improving the technique and determining the optimal position of the device. Using our technique one piezoelectric sensor was placed between the impeller and the chopper inside the pelletizing bowl right above the wet mass. Signals from the piezoelectric device were collected, analyzed and the spectrum of sound was evaluated. Previous investigations confirmed that close correlation could be found between the end-point determined by acoustic analysis and the end-point determined by impeller torque, however a slight difference could be observed between the models. We focused on the difference between these two methods in our experiments and tried to find the relationship taking into account the process factors [1-4].

EXPERIMENTAL METHODS

• Materials

Ibuprophen, α-lactose-monohydrate, microcrystalline cellulose (Avicel PH 101), and ethylcellulose were used to prepare pellets. Purified water was used as granulation liquid. Quality of all materials used in the experiments were Ph.Eur.

• Methods

Pellets were produced using a 1000 ml laboratory high-shear mixer (Pro-C-epT 4M8 Granulator, Belgium, Zelzate) with a three blade impeller and a chopper.

Pellets of 15 trials were prepared according to a central composite factorial design (Table 1). TableCurve® 3D v4.0 (Systat Software Inc., London, UK) was used to reveal the response surface from the polynomial equation calculated using the experimental design.

Table 1: Process factors in CCD

Factors with coded levels	-1	0	+1
A: Impeller speed (rpm)	1000	1250	1500
B: Chopper sped (rpm)	1000	1250	1500
C: Binder flow (ml/min)	8	9	10

RESULTS AND DISCUSSION

Analysis of Central Composite Design results the following equation of response surface calculating according to the impeller torque (IT):

$$Ep\ (IT) = 267.07 - 5.20A + 3.60B - 30.30C$$

Equation calculated according to the acoustic analysis (AA) is the following:

$$Ep\ (AA) = 270.33 - 6.90A + 7.60B - 31.00C$$

At both cases linear model was found to be significant ($P < 0.0001$, $P \leq 0.05$ was considered to be significant).

Comparing the coefficients of the equations above we can state that a slight difference could be observed, which could be due to the difference in the sensitivity of the applied methods. Equation below represents of the difference:

$$Ep\ (difference)\ (sec) = 3.26 - 1.70A + 4.00B - 0.70C$$

Equation above means that the higher chopper speed and lower impeller speed causes higher difference of time of end-points determined by applied two techniques in our experiments. Figures 1-2 represent response surfaces of difference.

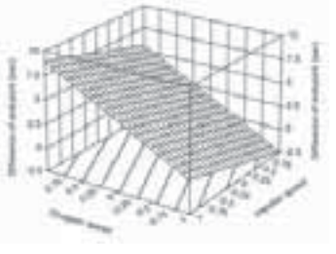

Figure 1: Effect of impeller and chopper speed on difference of end-point between the applied techniques.

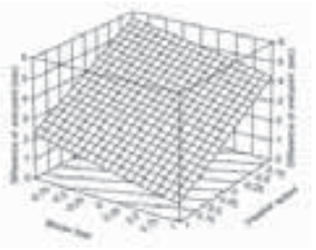

Figure 2: Effect of impeller speed and binder flow on difference of end-point between the applied techniques.

CONCLUSION

Experiments built according to a Central Composite Design confirm that comparing times of end-points of pelletizing determined by impeller torque and acoustic signal analysis are in close correlation, however a slight difference could be observed between the calculated coefficients of the response surface models at the two techniques. Our aim was to determine the trend of difference taking into account the process factors. Calculating this trend could serve important information for expert developer of high-shear granulator and also for the developer of a dosage form.

References

1. Briens, L., Daniher, D., Tallevi, A., 2006, Monitoring high-shear granulation using sound and vibration measurements, Int. J. Pharm. 331, 54–60.
2. Dévay, A., Mayer, K., Pál, Sz., Antal, I., 2006, Investigation on drug dissolution and particle characteristic of pellets related to manufacturing process variables of high-shear granulation, J. Biochem Biophys Methods. 69, 197-205
3. Levin, M., In: Levin, M., Editor, Pharmaceutical Process Scale-Up, 2002, Drugs and the Pharmaceutical Sciences Volume 118, Marcel Dekker, New York
4. Whitaker, M., Baker, G.R., Westrup, J., Goulding, P.A., Rudd, D.R., Belchamber, R.M., Collins, M.P., 2000, Application of acoustic emission to the monitoring and end point determination of a high shear granulation process, Int. J. Pharm. 204, 79–91.

PO090

Mechanical properties of pellets of different composition and preparation

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INTRODUCTION

Appropriate mechanical properties of pellets, i.e. their hardness and friability, are important characteristics to withstand subsequent processes such as coating, filling into hard gelatin capsules or compressing into tablets. Pellet hardness is usually measured using tablet strength testers equipped with a cell for pellet evaluation. However it is significantly dependent on pellet diameter, composition and manufacturing process [1]. Pellet friability can be determined by a number of different methods using various equipment (rotating drums, Turbula blenders, mixers or air stream apparatus) [2]. To increase the mechanical stress during these tests glass or steel balls are added to pellet samples. However parameters of the tests differ significantly in a number of added balls, rotating speeds and times or sample amounts.

In this experimental study pellet hardness and friability were tested, and the influence of pellet composition, preparation process and variables of friability test on pellet mechanical properties was observed.

EXPERIMENTAL METHODS

In this experiment, pellets of different composition and manufacture process were chosen (Table 1). All drugs and excipients were of pharmaceutical grade.

Crushing strength was tested in C 5 Hardness tester (Engineering Systems, England). Friability measurements were carried out in a friabilator Roche (Erweka TAR 10, Germany) using stainless steel drum to prevent pellet agglomeration due to electrostatic charge; 10, 100 or 200

Table 1: Composition of the samples, pellet preparation and size.

Sample	Composition (%)					Technology used for preparation	Pellet size (mm)
	DHCl[a)]	DNa[b)]	MCC[c)]	LM[d)]	PVP[e)]		
1	48.5	-	48.5	-	3.0	layering of DHCl on MCC cores	0.5 – 0.8
2	55.0	-	35.0	10.0	-	rotoagglomeration	0.8 – 1.0
3	48.5	-	17.0	31.6	2.9	layering of DHCl on LM/ MCC cores	0.8 – 1.0
4	-	-	100.0	-	-	Celphere®	0.5 – 0.71
5	-	10.0	35.0	55.0	-	rotoagglomeration	0.8 – 1.0
6	-	-	35.0	65.0	-	rotoagglomeration	0.8 – 1.0

Comments: [a)]diltiazem hydrochloride, [b)]diclofenac sodium, [c)]microcrystalline cellulose, [d)]lactose monohydrate, [e)]povidone

Table 2: Hardness and friabilities of prepared pellets

Sample	Pellet hardness (N)	Friability (%)				
		200/ 10/ 20*	100/ 10/ 20	10/ 10/ 20	100/ 5.5/ 36	10/ 5.5/ 36
1	0.87	3.16	1.56	0.50	1.85	0.71
2	1.67	2.62	1.49	0.35	1.64	0.47
3	2.84	1.91	0.82	0.29	1.04	0.29
4	3.41	0.38	0.24	0.19	0.26	0.24
5	3.61	0.26	0.22	0.14	0.24	0.19
6	4.89	0.18	0.09	0.07	0.17	0.13

* number of glass beads/ rotation time (min)/ rotation speed (rpm)

pieces of glass balls 4 mm in diameter, rotation speeds 20 or 36 rpm, and rotation time of 10 and 5.5 min, respectively. Despite of the different test conditions, friability of the samples was always measured after 200 rotations.

RESULTS AND DISCUSSION

Pellets of different size and hardness were chosen for this experiment to show the influence of method variables on the friability of brittle and hard pellets prepared by different techniques. All the samples exhibited similar residue moisture content. Table 2 indicates hardness and friability of pellet samples.

It is obvious that with increasing pellet hardness friability of samples decreases despite of the friability test conditions (Table 2). Harder pellets showed lower sensitivity to different friability test conditions (Figure 1). In addition pellets prepared using the drug layering technique generally showed higher sensitivity to friability conditions than pellets produced by a rotoagglomeration (samples 1 and 3, Table 2 and Figure 1). Furthermore when the inactive cores are compared, better mechanical properties were obtained for active pellets with LM/MCC cores than pellets started with only MCC cores (pellet hardness of 2.84 vs. 0.87 N, and friabilities 0.29 – 1.91 % vs. 0.50 – 3.16 N). This can be explained by different cores solubility and wettability. While MCC cores are insoluble in water, which was used as wetting agent for the layering, LM/ MCC cores were partly soluble due to high lactose content. For successful drug solution layering process it is necessary to wet properly the solid surface of the starter. Only at these conditions, droplets of the drug solution could spread all over the solid surface and create a continuous drug layer. As LM/ MCC cores are partly soluble in water, one might presume their surface is better wetted by the drug solution making the layering process more effective and the drug layer adhering more tightly to the core surface. This theory was supported by SEM images of layered pellets and also pellets after the friability test (data not shown).

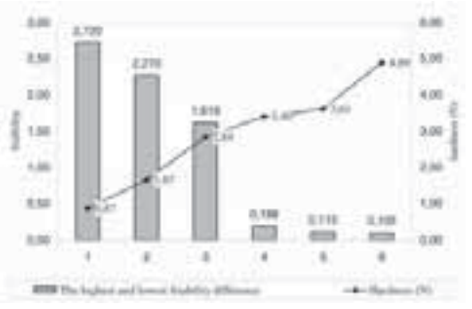

Figure 1: Sensitivity of different pellet samples to variable conditions of friability tests

Since limit values for pellet friability were published in the literature, i.e. less than 1.7 % [3], it is necessary to pay attention to the method used and its parameters. As shown in our experiment, different friability values of one sample can be obtained indicating either satisfactory or unsatisfactory friability values. Especially brittle pellets are very sensitive to the variables of friability method used.

ACKNOWLEDGEMENTS

This experimental work has been supported by the pharmaceutical company Zentiva a.s. Praha, Czech Republic. Cellets® were kindly donated by Syntapharm GmbH, Germany.

References

1. Podzceck F., Almeida S. M. Determination of the mechanical properties of pellets and film coated pellets using Dynamic Mechanical Analysis. Eur. J Pharm. Sci. 16: 209-214 (2002).
2. Schultz P., Kleinebudde P. Determination of pellet friability by use of an air stream apparatus. Pharm. Ind. 57: 323-328 (1995].
3. Vertommen J., Kinget R. The influence of five selected processing and formulation variables on the particle size, particle size distribution, and friability of pellets produced in a rotary processor. Drug Dev. Ind. Pharm. 23: 39-46 (1997).

PO091

The influence of water used as binder on the characteristics of pellets produced by extrusion and spheronisation

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INTRODUCTION

For some time, extrusion and spheroniastion as a process for the production of pellets has gained an increased usage dut to the interest in multiparticulate controlled-release oral dosage forms [1, 2].

In terms of size, shape, flowability and friability as important pellet properties, the production of pellets by extrusion/spheronisation requires the control of all formulation variables and of all process steps. The granulation/extrusion step is especially critical for the quality of the pellets [3, 4].

The aim of this study was to determine the optimal amount of binder necessary for the preparation of some support pellets with suitable physical, rheological and mechanical properties, designed to be loaded subsequently by spray-coating with active ingredient solutions or suspensions and to be film-coated.

MATERIALS AND METHODS

For the preparation of pellets, equal amounts of Avicel PH 101 and a-lactose monohydrate were mixed in a cubic Rotomixer. Different amounts of water (40, 60 and 80 g for 100 g of powder mix) were added over the resulting homogenous mixture for each formula, the liquid acting as a binder. The mixture was kneaded for a 15 minutes minutes.

The wet mass was immediately transferred to an extruder with a radial screen (Model 25 extruder, Caleva Process Solutions, Ltd., UK), where it was processed as extrudates, at 30 rpm.

Two charges of 100 g wet extrudates were brought in the spheronizer (Model 120 Spheronizer, Caleva Process Solutions, Ltd., UK) right after manufacture and processed untill spherical pellets were obtained. The spheronisation was performed at 750 rpm, for 5 and 10 minutes.

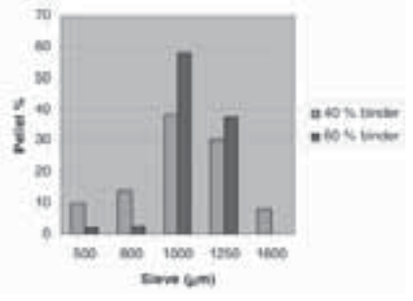
The resulting pellets were dried, untill reaching a constant weight (with a moisture content of less than 3 %).

In order to determine the influence of the amount of binder on the quality of the pellets, the following tests were carried out:

- *particle size distribution* – by sieve analysis (CISA® Sieve Shaker Med RP10, CISA Cedaceria Industrial, Spain);
- *morphology and elongation ratio* – by photo analysis (Olympus Camedia Master, Olympus Optical Co., Ltd., Japan);
- *bulk and tap density* – using methods described in the Romanian Pharmacopeia Xth edition, add. 2001 (VanKel Tap Density, VanKel Industries Inc., USA);
- *friability* – by the method described in the Romanian Pharmacopeia Xth edition, add. 2001 (Vankel Tablet Friabilator, Vankel Industries Inc., USA);

RESULTS AND DISCUSSIONS

The analysis of particle size distribution histograms indicates that the final products obtained have different characteristics. Thus, the addition of 40 g of binder to 100 g of excipient blend has lead to a longer spheronisation time and to a broader distribution range of the pellet size. A narrow distribution range of the pellet size resulted by using 60 g of distilled water to wet the powder mass.



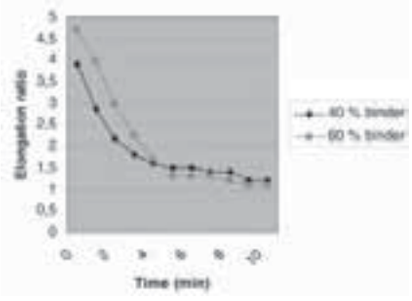
The influence of the amount of binder on the pellet size distribution

In both cases, a similiarity in the morphology of the pellets can be observed. After spheronization, all of the granules (extrudates) have acquired a shperical shape, with a smooth surface. Still, the formula with a higher amount of binder formed particles of a larger size. The elongation ratio, respectively the particle diameter, rapidly decreased at the begining of the spheronization process (in the first 2 – 3 minutes), and then it remained constant during spheronisation.

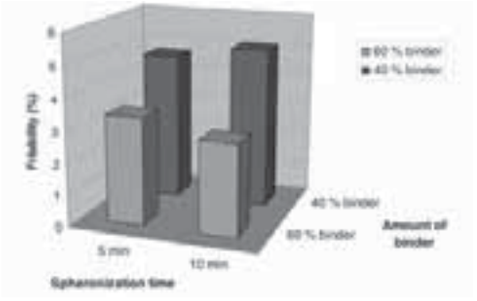
No significant differences can be observed regarding the density of the pellets. The small variations observed during the test is related to the difference in the disposition of the particles, as shown in the particle size distribution test.

The results of the friability test suggest a correlation between the mechanical strength and the interior pellet structure. The centrifugal force

that is manifested during spheronization decreases the porosity of the pellets and improves their compactibility, probably due to the higher content of water in the pellets, that leads to a stronger bonding of the particles and, thus, a low friability.



The correlation between the elongation ratio and the amount of binder



The correlation between friability, the amount of binder and spheronisation time

CONCLUSIONS

The amount of binder added to the formulation during the extrusion / spheronization process affected the pharmaceutical properties of the pellets.

In order to produce solid spheres, the extruded mass must be plastic enough to be rolled into a spherical shape, with a narrow distribution, a good flowability and a high mechanical strength.

References

1. Lindner H., Kleinebudde P., Use of powdered cellulose for the production of pellets by extrusion of pellets by extrusion / spheronisation, J. Pharm. Pharmacol., 1994, 46: 2 – 7;
2. Vervaet C., Baert L., Remon J. P., Extrusion / spheronisation: A literature review, iNt. J. Pharm., 1995, 116, 131 – 146;
3. Schmidt C., Kleinebudde P., The importance of the granulation step in pelletization by extrusion, spheronization, 2nd World Meeting on Pharmaceutics; Biopharmaceutics and Pharmaceutical Technology, Paris 25/28 May, 1998;
4. Leucuta S. E., Tehnologie farmaceutica industriala, Ed. Dacia, 2001.

PO092

Development and manufacture of pellets containing dry emulsions with application of fluid-bed technology

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INTRODUCTION

Dry emulsion can be defined as a dispersion of an oily droplets in the solid matrix. The active drug incorporated in emulsion is protected from degradation by the sun light and processes of oxidative decomposition. It also is well known that the active drugs of low aqueous solubility have higher bioavailability when administrated in the form of emulsion (1). Emulsions are formulated for virtually all major routes of administration. However oral emulsions have not been widely used due to the relatively large volume, liquid state, tendency to the instability processes such as creaming, flocculation, coalescence, and phase separation (2). Some of the instability problems can be solved by preparing the emulsions in form of dry emulsions.

To get a solid-state emulsion removing of the water phase of the original O/W emulsion has to be done by evaporation, lyophilisation or spray drying processes. By drying the aqueous phase is removed causing the carrier to form a matrix in witch the dispersed lipid phase is encapsulated. According to the literature data there have been several attempts to obtain dry emulsions(3). Some water-soluble or insoluble solid carriers should be added to the water phase. A variety of sugars and polyols can be used as a soluble carriers.

In our case pellets containing dry emulsion were manufactured by layering of the starting neutral pellet cores. The layering process was pre-

formed by spraying the previously prepared o/v emulsion using the Wurster process chamber, which is a fluid bed technology equipment. The aim of our work was to manufacture pellets with adequate layer thickness and therefore to ensure sufficient amount of lipid phase incorporated in the layer. The goal was to produce pellets with high amount of lipid phase because the content of a poorly water soluble drug which can be dissolved in a lipid phase is proportional to a lipid phase volume in pellets.

EXPERIMENTAL METHODS

Determination of ibuprofen solubility

Ibuprofen which is known as a poorly water soluble drug was used as a model drug substance. Solubility of ibuprofen was evaluated in Miglyol 812 (Salol, Germany) using method of addition. Samples of Miglyol solution of ibuprofen with concentrations rising per 5mg/ml were prepared until maximum concentration has been determined.

Emulsion preparation

Ibuprofen solution in concentration of 200 mg/ml was prepared by adding the ibuprofen to Miglyol 812 and then stirring for 30 minutes at room temperature. Additionally water solution of manitol (LEK, Slovenia) and sodium dodecil sulphate (Riedel-de Haen, Germany) was prepared while mixing. When all the substances were completely dissolved, we added the ibuprofen solution to the water phase and homogenised it with shear force homogenizer (Ultra-turrax T25, IKA-Labortechnic, Germany) for 5 minutes at 8000 RPM. The emulsion was heated to 37 °C and then homogenised in a two-stage valve homogeniser (Two stage APV-2000, USA) at 20 MPa for the first stage and 2 MPa for the second stage. Emulsion was homogenized three times to ensure a proper droplet size distribution.

Coating of pellets and characterization

Emulsions with manitol to ibuprofen ratio 2:1 in experiments A and B and ratio 2,5:1 in experiments C, D, E were prepared as described above. Each emulsion was sprayed with use of binary nozzle onto 200 g of starting pellet cores (Cellets 500, in experiment A and Cellets 200, Syntapharm, Germany in experiment C) using fluidized bed coater (GPCG-1,Glatt® GmbH, Germany, Wurster insert).

Processing parameters were the same in all experiments: mass of the starting cores, 200g; inlet air temperature, 55°C; spray rate, 5-10 g/min; spray nozzle diameter, 1,2mm. Variable process parameters are described in Table 1.

Table 1: Variable process parameters:

Exp.	$v_{(air)}$	$T_{(prod)}$	$P_{(atm)}$	$H_{(wi)}$
A	3,8	39	0.18	20
B	3,8	38-39	0.18	25
C	3,2	37	0.19	15
D	3,2	37	0.19	15
E	3,2	37	0.19	15

$v_{(air)}$ -inlet air velocity (m/s)
$T_{(prod)}$ -product temperature (°C)
$P_{(atm)}$ -atomizing air pressure (MPa)
$H_{(wi)}$ – position of Wurster insert (mm)

To get a sufficient layer thickness the so called stage layering had to be performed., Pellets produced in experiment A served as a starting cores in experiment B and coated pellets obtained from experiment C were used as a starting cores in experiment D and pellets manufactured in experiment D as an initial cores in experiment E. In case of single stage coating process the amount of manufactured pellets would exceed the capacity of the process Wurster chamber. At the end of coating process, the coated pellets were dried for 5 min.

Yield of the coating process was determined as a ratio of mass increase to the mass of dry material in emulsion sprayed onto pellet cores. Arithmetic mean diameters of pellets were calculated using the data obtained from sieve analysis.

RESULTS AND DISCUSSION

Maximum solubility of ibuprofen in Miglyol 812 was determined at concentration of 200mg/ml at 20°C.

Prepared emulsions were stable during the coating process. Determination of droplets distribution by laser difractometer (Mastersizer S, Malvern Instruments, GB) confirmed that there was no tendency of coalescence or flocculation of dispersed droplets. Calculated total yields for each experiment and mean diameters of layered pellets are listed in Table 2.

Table 2: Total yields and arithmetic mean diameters

experiment	total yield	arithmetic mean diameter
A	93,81 %	811 m
B	93,37 %	1169 m
C	95,40 %	445 m
D	94,68 %	746 m
E	94,86 %	1106 m

Figure 1: SEM micrographs section of pellets from experiment E at 140 X magnification (Zeiss Supra, 35 VP)

Figure 2: Surface of pellets from exp. E at 40 X magnification (Stereomicroscope Olympus)

Some problems have emerged during the experiments A and B associated with retention of pellets at the bottom of the Wurster process chamber, moderate agglomeration, and filter clogging. All those problems were successfully resolved in experiments C, D and E were the emulsion with higher portion of manitol was used. In Figure 1 we can see the dry emulsion layer of the pellet obtained with experiment E and the hollow region where the initial core had been. Surface and diameter of pellets manufactured in experiment E can be observed in Figure 2.

CONCLUSION

Fluid bed technology equipment (Wurster processing chamber) enables manufacturing of pellets with dry emulsion coating. The use of high pressure homogenizer is essential to produce an emulsion with suitable size distribution of dispersed phase. Process efficiency is in close relationship with the ratio of manitol to ibuprofen in Miglyol 812 solution, which was 2:1 in experiments A, B and 2.5:1 in expperiments C, D, E.

References

1. Chen Z., Davidson N. O.: Genetic Regulation of Intestinal Lipid Transport and Metabolism, Physiology of the Gastrointestinal Tract, 4th Ed., Academic Press, 2006: 1711-1734
2. Eccleston G. M. : Emulsions and Microemulsions. In: Swabrick J. C., Encyclopedia of Pharmaceutical Technology, 3rd Ed., vol 3, New York, 2007: 1548-1565
3. Fude Cui et al.: Preparation of redispersible dry emulsion using EudragitE100 as booth solid carrier and unique emulsifier, Colloids and Surfaces A: Physiochem. Eng. Aspects, vol 307,2007; 137-141

PO093

Optimization of melt pelletization in a high-shear mixer

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INTRODUCTION

Thermoplastic pelletization in a high-shear mixer belongs to the group of hot-melt technologies which represent an alternative to classical technological processes and offer a number of advantages. The process does not require the use of solvents and is therefore suitable for the formulation of moisture-sensitive active ingredients [1]. Moreover, the drying phase is eliminated and consequently the process is shorter and more economical. Chemically and physically versatile group of meltable binders also assures flexibility in the design of the pharmaceutical dosage forms.

The main limitation of hot melt technologies are high temperatures required which can cause degradation and oxidative instability of the ingredients. Another disadvantage is sensitivity of the process to changes in the formulation and process variables. [1-4]

The present study investigates the differences between two formulations of a poorly soluble model drug, prepared with two different binders, and the processes for the production thereof .

EXPERIMENTAL METHODS

Lansoprazole (Krka, Novo mesto, Slovenia) was used as a poorly soluble active ingredient. Magnesium hydroxide carbonate (Cognis, Düsseldorf, Germany) was used as a stabilizer. Lactose 450 mesh (α-lactose monohydrate; DMV, Veghel, The Netherlands) was used as a diluent. Each formulation was prepared using a separate binder: the macrogolglyceride binder Gelucire® 50/13 (Gattefosse, Saint Priest, France), and the poloxameric binder Lutrol® F68 (BASF, Burgbernheim, Germany); which differ in their melting point, viscosity and particle size. The melting behaviour of the binders was evaluated by a Mettler STAR^e SW 8.01, differential scanning calorimeter (Mettler, Schwarzenbach, Switzerland). Samples of about 4 mg were sealed in 40 L aluminium pans and scanned between 10°C and 70°C at a heating rate of 5 K/min. The viscosities of the molten binders were estimated by a Physica Rheolab MC 100 UM (Germany) at a shear rate of 95 s^{-1} and different temperatures.

The matrix pellets were prepared by hot melt pelletisation in a ProCept Mi-Pro high-shear mixer equipped with a double jacket for heating/cooling and a six-bladed impeller, with a bowl capacity of 250g.

Concentration of the two binders, mixing times and temperatures were varied according to the Box Behnken design plan.

At the end of the granulation process, the granules were cooled at room temperature and sieved through a 2.4 mm sieve to determine the amount of lumps. All experiments were performed in duplicates.

The total yield of the process was measured as the amount of pellets passing through the 2.4 mm sieve. Pellets in the range 0.500 – 1.400 mm were selected as the useful fraction.

The size distribution of obtained pellets was evaluated by sieve analysis, using a vibrating shaker and 7 sieves in the range of 0.250 - 2.00 mm (Prufsieb Jel 200, Hosokawa, Augsburg, Germany).

The shape and surface properties of selected pellets were investigated using an optical microscope (Stereomicroscope Olympus SZH10, Tokyo, Japan), equipped with a Sony DXC-107AP camera.

A field emission scanning electron microscope, FE-SEM SUPRA 35 VP (Carl Zeiss, Oberkochen, Germany), equipped with energy dispersive

spectroscopy Inca 400 (Oxford Instruments, Oxford, UK) was used for the investigation of the active ingredient's distribution within the pellets. The Box-Behnken experimental design and the response surface methodology were used to determine the influence of particular process parameters and binder concentration on the mechanism of pellet growth and their physical properties.

RESULTS AND DISCUSSION

The useful yield of the formulation in case of a low-viscosity binder (Gelucire® 50/13) was found to be mostly influenced by the concentration of the binder (Fig.1) although the influence of a single factor was hard to explain since both interactions and square factors were substantial.

This can be attributed to the growth mechanisms with distribution being predominant in the nucleation phase and steady growth in the consolidation phase.

Two interactions between the varied parameters were determined (a positive interaction between mixing speed and mixing time and a negative interaction between the mixing speed and binder concentration).

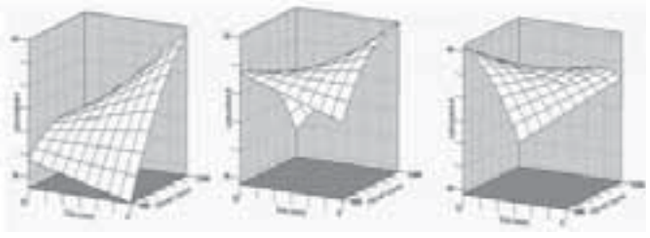

Figure1: The influence of mixing time (x-axis) and mixing speed (z-axis) on the useful yield (y-axis) at three different binder concentrations.

Although the viscosity of the binder was shown to be too high to achieve a controllable process in case of Lutrol® F68, massing time was identified as the variable that most influenced the pelletization process, while the concentration of the binder did not seem to have an influence on the useful yield (Fig.2). Both distribution and immersion mechanisms were present in the nucleation phase.

No interactions between the parameters were noted. The differences in the formulations during the pelletisation process were attributed to the differences in physical properties of the binders.

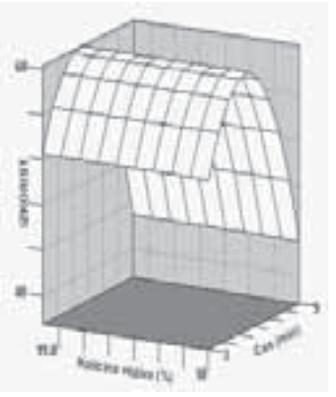

Figure 2: The influence binder concentration (x-axis) and mixing time (z-axis) on the useful yield (y-axis)

The aim of the optimization of the process was to minimize unwanted products (residual powders and oversized agglomerates – lumps). The optimal quantity of the binder and optimal processing parameters for pelletisation were identified within the studied intervals. The predicted response values obtained mathematically (by regression model) were also confirmed experimentally and the models were successfully validated, regardless of the binder used.

CONCLUSION

The results of the present work show that thermoplastic pelletization in a high-shear mixer is a simple and effective alternative to classical pharmaceutical methods. The process itself strongly depends on the thermal and rheological behavior of the binder used and is sensitive to the changes in process and formulation variables. This drawback can be overcome by identification of critical parameters, their optimization and careful control.

References

1. Schaefer T, Holm P, Kristensen HG. Melt granulation in a high shear mixer. *Drug Dev Ind Pharm* 1990; 16: 1249-1277.
2. Thies R, Kleinebudde P. Melt pelletisation of a hygrscopic drug in a high shear mixer. Part 1. Influence of process variables. Int J Pharm 1999; 188: 131-143.
3. Schaefer T, Holm P, Kristensen HG. Melt pelletization in a high shear mixer. I. Effects of process variables and binder. *Acta Pharm Nord*. 1992: 4: 133-140.
4. Voinovich D. Campisi B, Moneghini M, Vincenzi C, Phan-Tan-Luu R. Screening of high shear mixer melt granulation process variables using an asymmetrical factorial design. Int J Pharm 1999; 190: 73-8

PO094

Design of Granule Structure: The effect of the spatial distribution of the formulation Ingredients on API release

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ABSTRACT

A granule is a composite mixture of one or more type of primary solid particles held toghether by a binder, and represents an effective way to deliver solid pharmaceutical products. These products are either transiotry, existing only from one process step to another (e.g., feed for pharmaceutical tableting) or as the final product on its own. In both considerations the spatial arrangement of formulation ingredients within the product matrix is very important in regard to its visco-elastic response and API release pattern.

In our recent work [1, 2 & 3] we have established a novel methodology that can be used to control the spatial distribution of the API (or any other component of interest) within the granule structure by introducing API as solid dispersion with in-situ melt or aqueous polymeric binders. In this novel approach a binder particle, which is relatively coarser than the primary solids, serves as a nucleus for a single granule and the growth occurs by a gradual build-up of primary solids on the surface of the nucleus. As the binder particle melts, the liquid is drawn by capillary forces into the upper granular structure and thus develops a hollow core. A schematic illustration of granule formation with encapsulated API is shown in Figure 1.

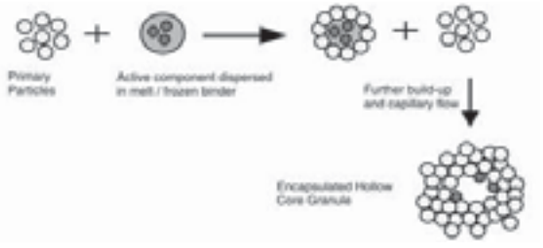

Figure 1: Schematic illustration of an encapsulated granule formation process by the immersion and layering mechanism.

In our proposed study we intend to take these findings a step further by examining the dissolution behaviour of the tablets compressed from pre-designed granules of two types, i) granules with model API randomly distributed in the structure matrix and ii) granule with API encapsulated in the central region as core.

We will also utilise the above mentioned novel approach to produce granules that have compartmentalised model reactive partilces. These granules will then be subjected to dissolution test to study the release behaviour of the API that would be produced in-situ as a result of the interaction between the reactive particles contained within the granule. Since granule properties profoundly influence the tablet dissoultion charactersitics, the idea of comparmentalised granules would offer a range of applications in the area where in-situ API are essential formulation or physiological requirements.

References

1. Ansari M.A., and Stepanek F. 2006, "Formation of hollow core granules by fluid bed in situ melt granulation: Modelling and experiments", International Journal of Pharmaceutics, vol. 321, pp: 108–116.
2. Ansari M.A., and Stepanek F. 2006, "Design of granule Structure: computational methods and experimental realization" AIChE Journal , vol 52, no 11, pp: 3762-3774.

PO095

Fluidized hot–melt granulation with macrogol 6000 and different fillers

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INTRODUCTION

Wet granulation in high shear mixer or in fluidized bed are the most common methods used for granulation. Alternative for wet granulation are dry methods: more traditional compacting in a roller compactors with subsequent milling and becoming popular melt granulation, based on agglomeration by use of a binder material (PEG, poloxamer, waxes, fatty

acids and triglycerides) that is solid at room temperature and softens and melts at higher temperature. Among the techniques used fluidized hot melt granulation (FHMG) is a still underdeveloped process [1, 2].

Significant advantage of melt granulation methods is that judicious choice of the granulation excipient may enable the formulator to manipulate the drug dissolution rate from the corresponding dosage form. In our study we have been testing application of FHMG for processing granules and tablets with carbamazepine (C) using as a binder macrogol 6000 (PEG 6000, 10% w/w) and different excipients as fillers.

MATERIALS AND METHODS

• Granulation

The following substances were used for preparation of granules: carbamazepine (micronized; Polpharma, Starogard Gdanski, Poland), macrogol 6000 (PEG 6000, Clariant, Burgkirchen, Germany) with the melting point 61.6ºC, lactose (Granulac 200 - Meggle, Wasserburg, Germany), mannitol (Pearlitol 200 SD, Roquette, Lestrem, France), calcium hydrogen phosphate – Di-Cafos (Chemisha Fabric, Budenhein, Germany), microcrystalline cellulose (MCC - Avicel PH 101 - Rettenmaier, Rosenberg, Germany).

The granulation (1 kg batches) was performed in a fluidized bed granulator (GPCG-3, Glatt, Dresden, Germany). The PEG 6000 (binder) content in all formulations was 10% (w/w). In placebo granules 90% (w/w) of the mass was a filler: MCC, lactose, calcium hydrogen phosphate, mannitol or 50:50 mixture of MCC and lactose. Granules with C contained 30%, 45% or 70% (w/w) of the drug and 60%, 45% or 20% (w/w) of a filler, respectively.
All powders were added to the fluidized bed and heated at 65ºC for 10 min. The granules were collected after cooling to 45ºC and were passed trough a 1.5 mm screen.

• Tabletting

Before tabletting 0.5% (w/w) of magnesium stearate, 1.0% (w/w) of colloidal anhydrous silica (Aerosil 200) and 2.0% (w/w) of crospovidone (Polyplasdone XL-10) were added to the granules. The tablets were compressed in a tablet press machine (C-50 - Kilian, Köln, Germany) with punches of 12 mm in diameter and the pressure adjusted to obtain the predetermined hardness (70 N). Tablets with C were also produced by wet granulation method.

• Analysis

The granules were analysed for: particle size distribution, density, compressibility index (Carr´s index), Hausner ratio and flowability. For tablets, thickness, hardness, friability and disintegration time were determined. Moreover dissolution rate of C was studied using sodium lauryl sulfate 1% (w/w) solution. Analysis of C in the dissolution medium was performed spectrophotometrically at the wavelength 280 nm.

RESULTS AND DISCUSSION

The FMHG process was successful for all fillers when C content was lower 30%. Above this content the granules adhered to the dryer's wall. For all fillers large portion of the granules (30–50%) was in the range 500-1000 μm. The granules with Di-Cafos were the most homogeneous since 90% of the particles were in the range 100-300 μm. The granules with C prepared with MCC:lactose were the least homogenous with 15% of particles bigger than 1000 μm and almost 10% of particles smaller than 50 μm.

Better flowability was observed for granules with 30% of C than for placebo granules with the same filler. Granules with lactose or Di-Cafos had better flowability and flow angle than other two types of granules. The Carr´s index <15% and Hausner ratio <1.18 for placebo granules and with 30% of C prove excellent and good flowability of FHMG product prepared with each filler. However, the flowability was poor when C content was 45% and higher. In Fig. 1 much better flow rate of granules prepared by FHMG than by wet granulation or compaction (the same composition) is demonstrated.

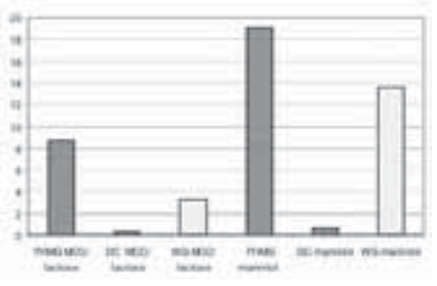

Figure 1: Comparison of flowability [g/s] of granules with MCC:lactose or mannitol prepared by FHMG, DC (compaction) and WG (wet granulation).

The tablets compressed from the granules exhibited a long disintegration time (30 min and more), however when Aerosil and crospovidone were added before tabletting, the disintegration time dropped to less than 5 min and 10 min, for placebo and tablets with C, respectively.

Tablets obtained from granules prepared by FHMG had lower values of disintegration time and friability than tablets produced from the granules prepared by wet granulation.

The results of the dissolution study of C from tablets are presented in Fig. 2. Tablets made from FHMG granules with mannitol or with MCC:lactose exhibit practically 100% of dissolution after 15 minutes. Corresponding tablets of the same composition but prepared with granules made by wet granulation have much worse dissolution profiles. Tablets with Di-Cafos and with lactose do not allow for fast release of C, even if the granules used for their production were prepared by FHMG.

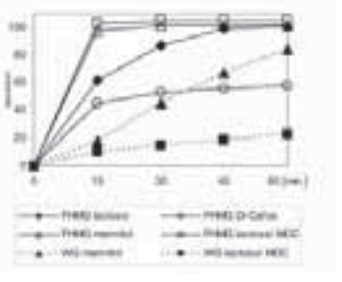

Figure 2: Dissolution profiles of carbamazepine (30% w/w) tablets prepared from granules obtained by FHMG process and wet granulation (WG).

CONCLUSIONS

FHMG is a suitable method for preparing granules with PEG 6000 (10% w/w) as a binder and MCC, MCC:lactose, mannitol or calcium hydrogen phosphate as fillers. The placebo granules or granules with C (up to 30% w/w) demonstrated good flowability and compressibility. Tablets prepared from the granules had satisfying characteristics regarding hardness, friability and disintegration time. Fast release of C was achieved from tablets with mannitol or MCC:lactose. The studies are continued and the results are similarly good for two other active substances i.e. ranitidine HCl and aciclovir. The stability studies are in progress.

References

1. Kidokoro, M. et al. Drug Dev Ind Pharm. 28, 67-76 (2002).
2. Gavin, M. et al. Chem. Eng. Sci. 60 3867–3877 (2005).
3. Ansari, M.A., and Stepanek F. 2008, "The evolution of microstructure in threecomponent granulation and its effect on dissolution", Particulate Science and Technology , vol 26, no 1, pp: 55-66.

PO096

Nonaqueos moisture activated dry granulation in a high shear mixer

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INTRODUCTION

Wet granulation is used most commonly in the pharmaceutical industry. A disadvantage of this method is, however, that a time-consuming and costly drying step is required. If dry granulation or direct compression is used as an alternative in order to avoid the addition of water, other disadvantages such as poor flowability, insufficient density properties, and poor content uniformity might occur.

A unique modification of dry granulation process was proposed by workers at Bristol Laboratories through the use of a process termed "moisture activated dry granulation" (MAGD). In this procedure, the binder is blended with the drug plus filler, a small amount of water is added (up to 4%), and then the mixture is blended thoroughly. Microcrystalline cellulose (MCC) is subsequently added to sorb the small amount of moisture present. No traditional drying step is involved.

Accordingly, in our work we developed new approach named Nonaqueos Moisture Activated Dry Granulation (NAMAGD) for tablets with high amount of active principle, which has very poor flowability and insufficient compression properties.

MATERIALS AND METHODS

Active principal (API) was obtained from MSN, India (d(0.9) = 10 μm; BCS IV). All other excipients (USP or Ph. Eur. grade) were used according to in-house agreement and purchase politics of Lek Pharmaceuticals d.d. (Sandoz group). The values in the parentheses are the geometric mean particle sizes of the distribution by volume, determined by a Malvern 2601Lc laser diffraction particle sizer (Malvern Instruments, UK). A 25 litre high shear mixer (Gral 25; Antwerp, Belgium) was used for the NAMADG process. The PEG-400 was added through one component nozzle mounted in the lid of the mixing bowl close to the periphery of the lid. The formulation used in the investigation is shown in Table 1.

API, isomalt, PEG-4000 and polyplasdone XL were dry mixed for 3 min (impeller speed 100 rpm). After that the PEG-400 was added at a constant flow rate of 20 g/min. A PEG-400 content of 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.5 or 8.0% (w/w) was used. The mixing was continued for 3 min after the end of the liquid addition (impeller speed 150 rpm; chopper – high speed). Talc and aerosil 200 have been added and mixing for another one min (impeller speed 80 rpm). These samples were sieved (0.75 mm) and were then mixed with laboratory blender for one min with mixture of magnesium stearate and pruv, which was sieved (0.3 mm) before use. Mixture of powders was used for compressing into briquettes using a rotary tablet press (Killian LX 18; briquettes were 400 mg in weight and 11 mm in diameter) in a controlled environment. After that, briquettes were grinding twice (Frewitt) through 2.0 mm and then 1.2 mm sieve. Afterward, the second part of isomalt, polyplasdone XL, aerosil 200 and talc were added to granulate, and mixed for 3 min. Magnesium stearate (0.3 mm) was finally blended for 1 min and mixture (dry granulate) was compressed into tablets using rotary tablet press (Killian LX 18; tablets were 1000 mg in weight; biconvex punches, capsule-shaped).

Table 1: Composition of tablets mixture.

Composition	Content [%]	Function
API	73.58	Active
Crospovidone (Polyplasdone XL)	4.75	Disintegrant
Macrogol 400 (PEG-400)	5.00	Binder
Macrogol 4000 (PEG-4000)	1.00	Binder
Isomalt (galenIQ721)	4.67	Filler
Magnesium stearate	1.00	Lubricant
Talc	1.50	Glidant
Colloidal silicon dioxide (Aerosil 200)	2.00	Glidant
Sodium stearyl fumarate (Pruv)	1.00	Lubricant
Magnesium stearate	0.50	Lubricant
Crospovidone (Polyplasdone XL)	2.00	Disintegrant
Isomalt (galenIQ721)	2.00	Filler
Colloidal silicon dioxide (Aerosil 200)	0.50	Glidant
Talc	0.50	Glidant

Compressibility, sieve analysis, mass variation, crushing strength and friability were done according Ph. Eur. protocols using standard laboratory equipment.

RESULTS AND DISCUSSION

Preliminary investigations were carried out with PEG-400 content of 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.5 or 8.0% (w/w). The selection of these PEG contents was based on former experiments for MADG model in which water contents of 2.0 to 4.0% were used. The effects of PEG content on flowability is shown in Figure 1.

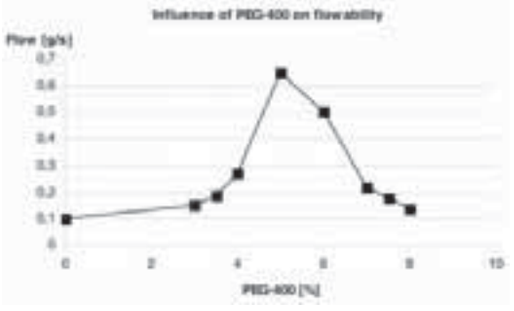

Figure 1: Effect of PEG-400 content on the flowability of powder mixture for briquetting.

The compressibility was used as a measure of the flow properties of the granulations. The lower compressibility the better flow properties. It was found by one-way ANOVA that the compressibility was affected by all the variables: formulation ($p < 0.001$), PEG-400 content ($p < 0.001$) and wet massing time ($p < 0.05$).

The mass variations were found to vary between 0.5 and 5.1% (RSD), and the best results were with PEG-400 in amount of 5%. It was impossible to make tablets containing more than 70% of voluminous API with unacceptable flowability and compressibility, with a crushing strength of more than about 50 N. Using combination of PEG-400/PEG-4000 in the ratio of 5/1 gives excellent properties of final tablet mass (1.60 g/s; 16.95%; 39.9°) with crushing strength of tablets between 100 and 120 N.

The friability was found to be affected by the crushing strength of the tablets ($p < 0.001$) and the formulation ($p < 0.001$). A higher crushing strength and an increasing content of PEG-4000 and isomalt reduced the friability. At the high level of crushing strength, the friability was lower than 0.1% with optimal PEG-4000 and isomalt content in formulation.

CONCLUSION

The present experiments confirm that the NAMADG technique can be used in a high shear mixer for producing granulations, which are suitable for tablet compression. Tablets with satisfactory characteristics could be produced on a double punch press from NAMADG granulations with isomalt, as well as a mixture of 5/1 of PEG-400 and PEG-4000. The content of PEG-400 has to be optimal in order to avoid flowability. If the PEG-400 content becomes too low or even too high, the flowability of the granules will be insufficient. Last, but not at least, NAMADG could be used for dry granulation of active principles which are voluminous, sticky, physically or chemically unstable in water, and with out acceptable flowability and compressibility.

References

1. Chen CM, Alli D, Igga MR, Cyeisler JL. Drug Dev Ind Pharm 16: 379 (1990).
2. Christensen LH, Johansen HE, Schaefer T. Drug Dev Ind Pharm 20: 2195 (1994)
3. Inghelbrecht S, Remon JP. Int J Pharm 171: 195 (1998)
4. Swarbrick J, Boylan JC (Ed.). Encyclopedia of Pharmaceutical Technology 4th ed. Marcel Dekker Inc., New York, USA, 1988, pp 102-104.

PO097

Effect of plasticizer content and degree of neutralization on gastro-resistance of HPMCAS coated pellets

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BACKGROUND AND OBJECTIVE

Hypromellose acetate succinate is cellulose derivate with a pH dependent solubility. Solubility in acidic media is low and gradually increases in neutral and alkali media. Due to these properties it is used as an enteric coating agent. It can be applied as an organic or aqueous dispersion. Due to environmental reasons an aqueous dispersion is preferred.

When using an aqueous suspension the main drawback is nozzle clogging which is caused by precipitation of polymer and plasticizer. To avoid precipitation coating suspension has to be cooled below room temperature. Another solution is neutralization of the polymer which is achieved by adding ammonia solution to the polymer suspension (1).

The influence of various formulation and process parameters including plasticizer content and coating level was investigated by Siepmann et. al (2). However these studies were made using aqueous polymer suspension.

The objective of this work was to study the influence of formulation parameters such as plasticizer concentration and the pH of HPMCAS coating dispersion on gastro-resistance of coated pellets. Additionally films of different polymer dispersions were prepared and glass transition temperature was measured with differential scanning calorimetry.

MATERIALS AND METHODS

Preparation of the samples: Pellets already coated with model drug layer, were coated using a laboratory scale fluid bed dryer with Wurster insert, Mini Glatt (Glatt GmbH). Coating dispersions were composed of hypromellose acetate succinate (AQOAT-LF, Shin-Etsu Chemical, Japan), triethyl citrate (Vertellus Performance Materials Inc., USA) as plasticizer, sodium dodecyl sulfate (Cognic Deutchland GmbH, Germany) as wetting agent, talc (Luzenac Val Chicone SPA, Italy) as anti-tacking agent and 25 % ammonia solution (Merck, Germany) as neutralizing agent.

Polymer films were cast from coating dispersions that were poured into a petri dish and dried at 40 °C for 3 hours to form a film. Films were then additionally dried in vacuum drier for 72 hours at 40 °C and pressure 230 mbar.

Gastro-resistance test: The test was performed by USP 1 apparatus (500 mL of 0,1 M HCl). After 2 hours pellets were transferred to alkali buffer solution and dissolved. The concentration of the model drug was determined using UV Spectrophotometer.

Thermal analysis: Polymer films were heated using conventional differential scanning calorimetry (DSC 822, Mettler Toledo) in non-hermetically closed Al-pans at a nitrogen purge of 40,0 mL/min. DCS analysis was performed using a three step temperature program. First step was heating from 20,0 -120,0 °C at a heating rate of 10 °C/min. Second step was cooling from 120,0-20,0 °C at a cooling rate of 10,0 °C/min. Third step was heating from 20,0-170,0 °C/min at a heating rate of 20 °C/min.

RESULTS AND DISCUSSION

Dispersions were neutralized by addition of fixed amount of ammonia solution to obtain fully neutralized dispersion of the polymer. Pellets were coated with two different coating levels. At first pellets were coated with 25 % of polymer per dry mass of pellets. Results of gastro-resistance tests show no significant differences in gastro-resistance of batches coated with dispersions containing different amounts of plasticizer. In the next step the coating level was reduced to 12,5 % of polymer per dry mass of pellets. Again no significant differences in gastric resistance were obtained among batches of pellets (Fig. 1).

The influence of polymer neutralization expressed as pH of the coating dispersion on gastro-resistance was studied in the next step. The 12,5 % coating level of polymer per dry mass of pellets was used. The amount of the plasticizer was 20 % relative to polymer. Results show that the degree of neutralization has a profound effect on gastro-resistance in pH rage between 5 and 6 (Fig. 2) Gastro-resistance increased with rising degree of neutralization up to pH 5.5, where it leveled of.

Thermal analysis of polymer films with DCS showed that the neutralization alone lowers the glass transition temperature (T_g) in comparison to non-neutralized polymer (Table 1). The combination of neutralization and addition of the plasticizer lowers T_g of polymer film more effectively than combination of non-neutralized polymer and plasticizer.

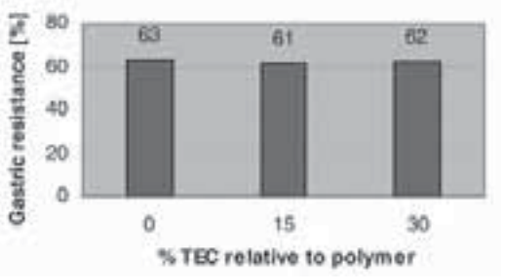

Figure 1: Influence of plasticizer content (12,5% coating level)

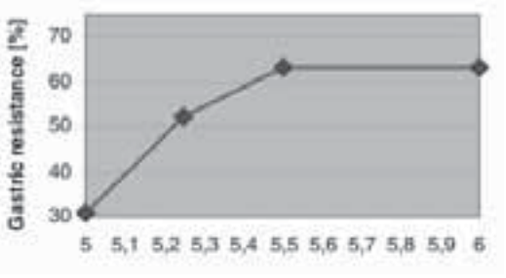

Figure 2: Influence of degree of neutralization on gastro-resistance of coated pellets

Table : Glass transition temperature of different polymer films

Sample	Neutralisation	Amount of plasticizer relative to polymer [%]	Glass Transition Temperature [°C]
Polymer (powder)	/	/	112,1
Film 1	Yes	0%	106,9
Film 2	Yes	15%	53,9
Film 3	Yes	30%	39,9
Film 4	No	20%	72,7

CONCLUSIONS

Pellet coating with neutralized HPMCAS was found to be a good alternative to classical suspension coating with non-neutralized polymer. It eliminates nozzle clogging without dispersion cooling. Also pellets with suitable gastro- resistance were obtained. Thermal analysis showed that plasticizer addition lowers T_g of polymer films more effectively when neutralized polymer is used compared to non-neutralized polymer.

References

1. McGinity J W. Aqueous Polymeric Coatings for Pharmaceutical Dosage Forms. Marcel Dekker, Inc., New York 1997
2. Siepmann F, Siepmann J, Walther M, MacRae R, Roland Bodimeier. Aqueous HPMCAS coatings: Effects of formulation and processing parameters on drug release and mass transport mechanisms. European Journal of Pharmaceutics and Biopharmaceutics 63 (2006) 262-269

PO098

Influence of eudragit NE concentration on the layering efficiency of carbamazepine-containing pellets obtained by powder layering

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INTRODUCTION

Carbamazepine is an antiepileptic drug widely used as monotherapy for people with partial or generalized onset tonic-clonic seizures and it is also usefull in tabetic pains [1]. The clinical problems due to the carbamazepine pharmacokinetics (half-life, conversion in CBZ-10,11-epoxide activ metabolit, enzyme-inducing action) could be at least partly resolved by controlled and extended-release drug terapy.

Multiple-unit systems ussually consist of pellets and they represent a comon way of obtaining well-controlled regulation of the drug release rate from oral drug formulations.

Polymethacrylates known under the trademark Eudragit could perform a dual function: delaying drug release and binder for pellets construction. Wet granulation is a verry effective way of producing controlled-release matrix with aqueous Eudragit dispersion. Eudragit NE formes a plymeric films, water insoluble, low permeabil, pH independent swelling due to its neutral structure. It is suitable for highly flexible matrix structure formation and doesn't require plasticizer. It is well suited for pelletizing in the coating pan for aglomeration of the spinkled particles and for fixing them to the core surface. The spinkled powder particles are bound to the surface of the cores by the capillary forces of the sprayed liquid droplets. The pellets formed are stabilized by the film that evolves from the polymer dispersion during the final drying phase. The polymer matrix normally slows down drug release especially if the active ingredient does not dissolve easily, the case of carbamazepine. In such of case, the presence in the matrix of soluble particles with hydrophilic groups (e.g. polyvinylpirolidone) may be necessary for the water penetration [2].

The aim of our study was to determine the infuence of Eudragit NE concentration on the the powder layering efficiency of carbamazepine - containing pellets obtained into the coating pan.

EXPERIMENTAL METHODS

Materials

Carbamazepine (Vimspectrum, Romania); sugar nonpareils 315-500 μm (laboratory products); Eudragit NE 30D- aqueous dispersion (Degusa, Germany); Kollidon K30 (Merck, Germany); Aerosil 200 (S&D Chemicals, United Kingdom).

Pellets preparation

Pellets were prepared by powdering layers of powder on sugar nonpareils in a conventional coating pan (Erweka, Germany).

Excipients of powder layering composition were mixed for 20 min. After mixing, the power mixture was sieved through a 120 μm screen and mixed again for 20 min. Binder solution was distilled water or was obtained by diluting Eudragit NE with water.Binder solution was sprayed on the moving nonpareils and the powder adition was started after a 2 min lag time of the binder solution. The loaded pellets were dried in a coating pan with a flow warm air (50 °C) for 15 min then in an oven at 40 °C for 24h.

The pellets were sieved using a set of sieves with different apertures (2000 μm, 1600 μm, 800 μm, 500 μm şi 315 μm 120 μm) The mean diameter was estimated as mean value.

Powder layering efficiency determination

Powder layering efficiency (PLE) was calculated by dividing the actual drug (Cr) content by the theoretical drug content (Ct) and multiplying by 100. The actual drug content was determined by spectrophotometrical (UV, at 285 nm) assay of the drug in pellets. The theoretical drug content was calculated by dividing the amount of drug present in the layering powder by the sum between charge loads of nonpareils and the charge of powder layering composition used [3]

RESULTS AND DISCUSSION

Table 1: Pellets compositions

Substance /prepared pellets	Formulation (g)			
	I	II	III	IV
Sugar nonpareils	17,50	17,50	17,50	17,50
Powder layering CBZ (33.75 g) Kollidon K30 (1,05g) Aerosil 200 (0,17 g)	34,97	34,97	34,97	34,97
Eudragit NE	-	0,3	0,6	1,8
Prepared pellets				
• calculated quantity	52,47	52,77	53,07	54,27
• obtained quantity	52,31	52,60	52,87	50,00

Table 1: Analysed products

Pellets product		Separated quantities (%)			
mean diameter (μm)	name	F I	F II	F III	F IV
1800	dim	6,6			
1200	dim I	87,6	76,0	68,9	82,8
650	dim II	5,8	20,0	27,2	10,3
407.5	dim III		4,0	3.9	6,9
Eudragit NE %		0	0,57	1,13	3,23

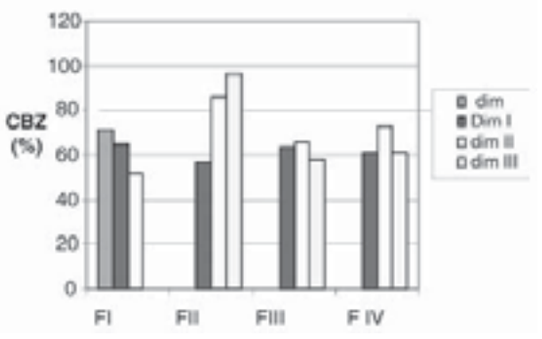

Figure 1: Carbamazepine pellets contents

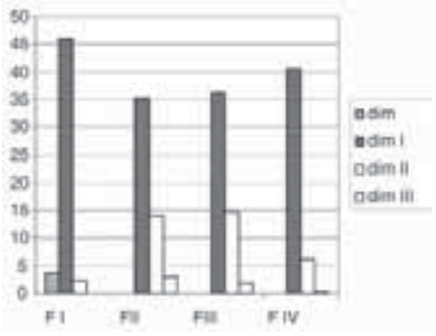

Figure 2: Powder layering efficiency(%)

Powder layering efficiency is corellated with the carbamazepine contents determined for the analysed products: dim II and dim III fractions of the pellets containing a large amounts of carbamazepine have lower powder layering efficiency which means an unbuilt structure af these small particles.

CONCLUSION

The formulation for pellets (composition and ratio) was judicious chosen, obtaining the following quantities of pellets (650-1200 m): F I- 87%; F II- 76%; F III- 69%; F IV- 83%.

Eudragit NE presence determines variations of powder layering efficiency depending on its concentration. Carbamazepine contents are in the range of 56-64%.

References

1. Cristea A.N. and all. Tratat de Farmacologie ed.I. Ed. Medicala, Bucure_ti: 121-131 (2005).
2. Practical course in film coating of pharmaceutical dosage forms with Eudragit. Pharma Polymers, Damstadt, Germany: (2001).
3. Tomuta I., Leucuta S.E. Use of experimental design for identifyng the most important formulation and technological variables in pelletization by powder layering. J. DRUG DEL. SCI. TECH., 14 (1): (2004)

PO099

Study on dissolution profile stability of pellets coated with ethyl acrylate methyl methacrylate copolymers

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INTRODUCTION

The dissolution profiles of products, where polymer coatings are responsible for the modification of release can sometimes show significant changes during storage. Provided that all the process parameters were proper, there are still numerous factors which influence the stability. The alteration of the release can be caused by the variation in the polymer structure (polymer relaxation) [1], the possible migration or disappearance of the plasticizer or the interaction between the polymer and the other excipients of the coating layer [2]. Moisture content of the product is also a critical factor in facilitating the occurrence of changes. The drug release behaviour of dosage forms coated with ethyl acrylate methyl methacrylate copolymers may show alterations in short term stability tests when using additives (pore-forming excipients or glidants) to improve the formulation performance.

The aqueous dispersion of the neutral copolymer Eudragit NE® 30D is used to provide a pH-independent release and has also been used in combination to provide zero–order kinetics [3]. Since the copolymer has a low minimal film forming temperature and the coating can become sticky at a relatively low temperature, it is advantageous to use an antitacking agent in the formulation. However, the quantity of this additive can increase the tendency of brittleness, lead to an imperfect coating layer and an unstable drug release.

The instability of Eudragit NE® containing nonoxynol-100 (1.5%) as an emulsifier has been reported by several authors to be caused by the crystallization of the emulsifier [4].

The aim of the study was to evaluate this polymer in coating technique studying its storage stability under different environmental conditions (e.g. temperature, relative humidity), compare the data of polymer free films and the pellet product, as well as to examine the effect of the talc level on the dissolution profile stability.

EXPERIMENTAL METHODS

• Preparation of Pellets

The batches of 300 g of pellets were prepared in a Stephan UMC 5 Electronic high shear mixer (Stephan, Germany). The active ingredient was theophylline (Ph. Eur. 5.) (Hungaropharma, Hungary), as a filler we applied microcrystalline cellulose (Avicel PH 101, FMC Biopolymers; Germany). Demineralized water was used as the granulating liquid. The conditions of pelletization are summarized in Table 1.

Table 1: Parameters of pelletization

Process	Rate (rpm)	Time (min)
Homogenization	1200	10
Spraying, wetting	900	15
Spheronization	900	10

The coating process was carried out in a fluid bed apparatus (Aeromatic Strea I, Aeromatic-Fielder AG, Switzerland) by bottom spray method to achieve a 5 w/w % dry polymer concentration. The pellets were coated with Eudragit® NE 30 D (Evonik Röhm GmbH, Germany), containing talc powder,< 10 μm (Sigma-Aldrich Chemie GmbH, Germany) as an antitacking agent in different ratios (100%, 50%, 0% calculated on dry polymer content)

• Preparation of free films

The polymer dispersions have the same composition and preparation method as seen above. The free films were sprayed on a teflon plate in a Glatt GC250 coater (Truttel Switzerland) with a spraying rate of 3.3 g/min and pressure of 1.5 bar. The inlet air was set 32 °C and the outlet air was 29 ± 2 °C. The dry films were carefully removed from the plate and kept at room temperature (25 °C ± 2 °C) for 3 days prior to stability testing.

• Stability Tests

The stability tests were carried out using a Sanyo PSC 022 stability chamber and hygrostat chambers (containing supersaturated solutions of sodium nitrate, sodium chloride and sodium bromide (Hungaropharma, Hungary) in sealed or open containers at different conditions. The relative humidity (R.H.) and temperature were varied as follows: 75% R.H. at 40 °C; 65% R.H. at 20 °C; 55% R.H. at 30 °C. The samples were tested after 1 day, 2 and 4 weeks of storage.

• Water Absorption of Pellets

The water absorption was checked by the weight increase of the pellets at an analytical balance (Sartorius BA110S, Germany) and also determined by Karl-Fischer titration using a Metrohm Titrino 787 KF Titrator (with Polytron PT 1600E homogenizer).

• Shape and Size Analysis

The moisture absorption of coated pellets was also studied on the diameter change of coated beads by image analysis. It was conducted on samples of 200 pellets using a stereomicroscope (SMZ 1000 type, Nikon, Japan) and the software Image Pro Plus 4.5 (Media Cynerbetics, USA).

• Dissolution Studies

The drug release tests were carried out in 900 mL demineralized water by using USP basket method with 100 rpm at 37 ± 0.5°C temperature (Hanson SR8-Plus™ Dissolution Test Station, Hanson Research, USA). The concentration of released theophylline was measured spectrophotometrically (λ= 273 nm, UNICAM UV/VIS Spectrometer UV2, UNICAM Ltd., UK). The dissolution curves were compared by Weibull-function, similarity and difference factors (f_2 and f_1) [5;6]

RESULTS AND DISCUSSION

The drug release shows the influences of the talc concentration variations (Fig. 1).

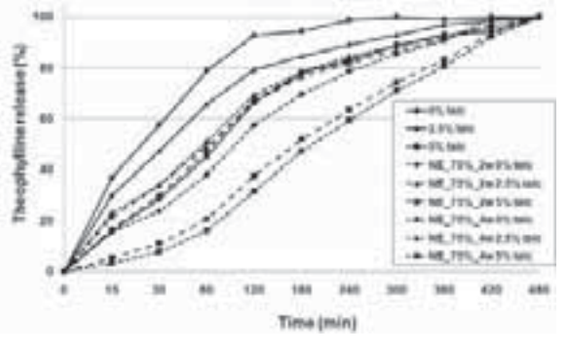

Figure 1: The effect of storage on the release of theophylline pellets after 2 and 4 weeks of storage

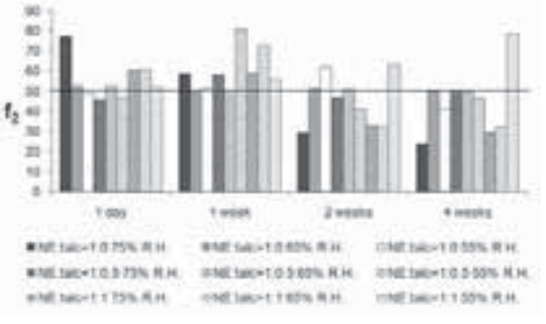

Figure 2: The similarity factors of the different release profiles

The calculation of similarity factors of the dissolution curves illustrates the impact of higher humidity and elevated temperature on the stability of the film coating layer (Fig. 2).

CONCLUSION

The amount of antitacking agent had significant effect on the dissolution profile of coated pellets. Development of coated multiparticulates requires the optimization of composition of the film-coating dispersion re-

garding additives, where stability tests and evaluation of dissolution curves during the preformulation phase are of great impact.

ACKNOWLEDGEMENTS
The authors are grateful to Evonik Röhm GmbH for the generous supply of Eudragit NE® and are also grateful to FMC Biopolymers for providing Avicel for the study.

References
1. Zelkó R., Orbán Á., Süvegh K.: J. Pharm. Biomed. Anal. 40: 249-254 (2006)
2. Petereit H.U., Weisbrod W.: Eur. J. Pharm. Biopharm. 47: 15-25 (1999)
3. http://www.pharma-polymere.de/pharmapolymers/en/downloads
4. Lin A. Y., Augsburger L.L.: AAPS Pharmsci 3 (2) article 14 (2001) (http://www.pharmsci.org/)
5. Langenbucher F.: J. Pharm. Pharmacol. 24: 979-981 (1972)
6. Moore J. W., Flanner H. H.: Pharm. Tech. 20: 64-67 (1996)

PO100

Effect of colloidal silicon dioxide and magnesium stearate on the lubrication coefficient in the compression process

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INTRODUCTION

During development of tablets instrumentation of presses plays an important role in the optimization of formulation. The compressibility can be characterized by experimental compression force/displacement data [1]. The densification process and the compaction behavior of the powder mixture depend both on the characteristics of drug and applied tableting agents. However, only few data are available on the interactions between different excipients used in parallel [2, 3].

In tablet composition, lubricants are added to reduce friction between the tablets and die wall during compaction and ejection, glidants are used to increase the flowing properties of the granules [4]. The effect of different levels of magnesium stearate (0.75-1.25 w/w%), colloidal silicon dioxide (0.1-0.3 w/w%) and lubrication time (5 and 10 min) on picking during process-scale up was earlier investigated [5]. It was found that colloidal silicon dioxide interacts with magnesium stearate and affects lubrication efficiency of magnesium stearate. Lack of lubricity or lubrication efficiency was manifested as picking in the tablets. 0.3% of colloidal silicon dioxide in the composition resulted more picking than 0.1%. Therefore lower amount of colloidal silicon dioxide was recommended to increase the lubricity of magnesium stearate. Johansson and Nicklasson reported that colloidal silicon dioxide interacts primarily with the free fraction of the lubricant which is then not available for lubrication of the die wall or further coverage on the surface of the powder blend [6].

A model formulation, comprising microcrystalline cellulose as direct compressing agent, was used to assess the influence of the glidant colloidal silicon dioxide in higher amount than earlier investigated, which is needed in case of active compounds with high dose and poor flowability properties. The objective was to characterize compression properties of the composition related to lubrication as well as to detect interaction between the different excipients.

EXPERIMENTAL METHODS

Materials

Model active ingredient was supplied by Gedeon Richter Plc. (Budapest, Hungary), microcrystalline cellulose (Vivapur 102) from JRS Pharma (Weissenborn, Germany), colloidal silicon dioxide (HDK N20) from Wecker Chemie (Burghausen, Germany), magnesium stearate from Faci Spa. (Carasco, Italy) were used as supplied.

Experimental design and tablet compression

The active ingredient, microcrystalline cellulose and colloidal silicon dioxide (where more than 0%) were blended in a container blender (Erweka AR 400, double cone shape container, (Erweka. GmbH, Heusenstamm, Germany) for 5 minutes with 20 rpm, than magnesium stearate was added and blended for further 3 minutes with 20 rpm. The batch size was 150 g. The amount of magnesium stearate was constant 1 w/w%. Lubrication coefficient as a typical parameter of lubrication properties was calculated to find the effect of the glidant and lubricant on the compression process.

The factors were the amount of colloidal silicon dioxide (0 w/w%; 0.5 w/w%; 1.0 w/w%) and the compression force (about 5 kN, about 9 kN, about 13 kN). The compression forces were slightly different from the targeted values but the exact compression forces were used for calculations. Central composite, face centered design was applied to determine the significant effects on the evaluated response parameters using Statistica software (StatSoft Inc., Tulsa, USA).

Tablet press instrumentation

A single punch eccentric tablet press (Type TM 20, Diaf, Copenhagen, Denmark) was used to produce tablets with the diameter of 10 mm and weight 250 mg. The instrumentation involved strain gauges (KMT-LIAS-06-3/350-5E type, Kaliber Ltd., Budapest, Hungary) on both the upper and lower punches. The displacement was measured by a magnetic sensor (Limes L2 type, Kübler GmbH, Villingen-Schwenningen, Germany) with a resolution of 5 μm and signaling at 1 s pulse interval. All signs were transferred through cables to the data recorder (USB-6210, National Instruments Corp., Austin, USA) involving signal conditioning.

RESULTS AND DISCUSSION

Figure 1 shows an example of the measured upper and lower punch compression forces and displacement profiles as a function of time measured at different compression forces.

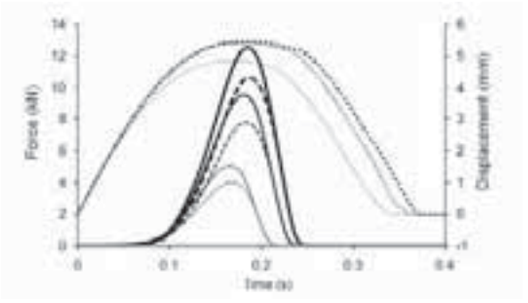

Figure 1: Upper and lower punch compression forces and upper punch displacements as a function of time (upper force: solid line; lower force: dashed line; displacement: dotted line)

The lubrication coefficient as R-value was calculated as the ratio of lower and upper maximum compression forces [7].

The statistical evaluation showed that the colloidal silicon dioxide and compression force together had significant effect on the lubrication coefficient. ($p<0.05$)

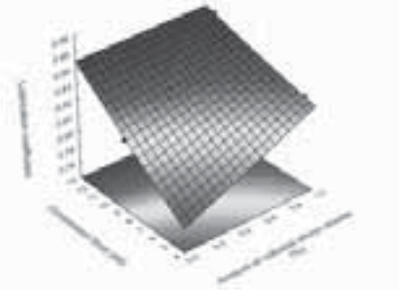

Figure 2: Response surface of lubrication coefficient as a function of compression force and the amount of colloidal silicon dioxide

Figure 2 demonstrates the calculated lubrication coefficient values in the examined range of 0-1.0 % colloidal silicon dioxide and 5-13 kN compression force. The lubrication coefficient was increased with the compression force and the tablets had higher lubrication coefficient as the amount of colloidal silicon dioxide increased. The results suggest that the interaction between magnesium stearate and colloidal silicon dioxide depends on the applied compression force and level of lubricant.

CONCLUSION

The lubrication coefficient was significantly influenced by both compression force and amount of glidant. In the presence of 1% magnesium stearate, higher amount of colloidal silicon dioxide resulted in better compression force transmission, especially when low compression forces were applied.

References

1. Antikainen, O., Yliruusi, J. Determining the compression behaviour of pharmaceutical powders from the force-distance compression profile. Int J Pharm. 252: 253-261 2003.
2. Busignies, V., Leclerc, B., Porion, P., Evesque, P., Couarraze, G., Tchoreloff, P. Compaction behaviour and new predictive approach to the compressibility of binary mixtures of pharmaceutical excipients. Eur J Pharm Biopharm. 64: 66-74. (2006)
3. Larhrib, H., Wells, J.I. Compression speed on polyethylene glycol and dicalcium phosphate tableted mixtures, Int. J. Pharm. 160: 197-206 (1998)
4. Zuurman, K., Van der Voort Maarschalk, K., Bolhuis, G.K. Effect of magnesium stearate on bonding and porosity expansion of tablets produced from materials with different consolidation properties. Int. J. Pharm., 179: 107-115 (1999)
5. Sabir, A., Evans, B., Jain, S.; Formulation and process optimization to eliminate picking from market image tablets. Int. J. Pharm. 215: 123-135 (2001)
6. Johansson, M.E., Nicklasson, M., Influence of mixing time, particle size, and colloidal silica on the surface coverage and lubrication of magnesium stearate Pharmaceutical Technology: Tabletting technology, vol.1. Halstead Press, New York, pp. 43-50 (1987)
7. Celik M. Overview of compaction data analysis techniques. Drug Dev. Ind. Pharm. 18: 767-810 (1992)

PO101

Influence of roll pressure during roll compaction on the granule- and tablet properties produced with paracetamol/microcrystalline cellulose-mixture

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INTRODUCTION

Roll compaction is a widely used dry granulation method. It can be especially suitable for moisture or heat sensitive drugs, because this technique requires no liquid binder and drying step. In roll compaction process the powder is fed between two counter-rotating rolls and compacted to dense ribbons. The produced ribbons are subsequently broken into granules. In most cases roll compaction is performed prior to tableting.

There are several process factors affecting the properties of produced granules by roll compaction and the resulting tablets: for example, roll pressure, roll speed, roll gap, and the speed of powder feeding can be

the critical parameters. The aim of this study is to investigate the influence of roll pressure during roll compaction on the properties of granules and tablets prepared with paracetamol/microcrystalline cellulose (1:1) mixture. Paracetamol is well known as a poorly compactable drug. Due to this characteristic the production of paracetamol tablets is almost exclusively by wet granulation [1]. For this reason, paracetamol was chosen as a model drug for roll compaction and the effect of roll pressure on the granule properties and the tensile strength of tablets was investigated.

EXPERIMENTAL METHODS

• Roll compaction

1:1 powder mixtures were prepared with paracetamol (Sandoz)/ microcrystalline cellulose (Avicel PH101, FMC, USA) and compacted at various roll pressures (0, 5, 10, 20, 30, 50, and 100bar) by roll compactor (Chilsonator IR 220, Fitzpatrick, Belgium) equipped with smooth rim rolls. The roll speed was set to 3 rpm. The produced ribbons were broken with 1.3mm screen sieves (FitzMill, Fitzpatrick, Belgium).

• Characterization of powder and granules

Particle size distributions were determined using laser diffraction (Mastersizer, Malvern Instruments, UK). Bulk- and tapped densities were measured in triplicate according to European pharmacopoeia, and the true densities were determined with a helium pycnometer (AccuPyc, Micromeritics, USA). The hausner ratio and the compressibility index were calculated based on bulk- and tapped density.

• Compaction of tablets

Flat-faced tablets of 10mm diameter and 400mg of mass were compacted at 20kN by tablet compaction simulator Presster™ (MCC presster, Metropolitan computing corporation, USA). The simulated machine was Korsch PH 336 of 36 stations. The press speed and the dwell time were set to 10800 TPH (Tablets Per Hour) and 118.3ms, respectively.

• Characterization of tablets

The breaking force of tablets was measured using a tablet hardness tester (Tablet tester 8M, Dr. Schleuniger Pharmatron, Mancheter). The tensile strength of tablet was calculated using equation (1).

$$\sigma = 2F/\pi hD \qquad (1)$$

In eq. (1), σ is the tensile strength (MPa), *F* the breaking force (N), *h* the thickness (mm), and *D is* the diameter (mm) (Fell and Newton, 1970).

RESULT AND DISCUSSION

As shown in figure 1, the bulk- and tapped densities of granules were increased after roll compaction. As expected, with increasing roll pressure the density was increasing too. True densities were also slightly increased by roll compaction. The hausner ratio and the compressibility index of granules were in the range of 1.17~1.26, and 11~21 respectively, which indicate acceptable flowability.

The mean particle size of granules was increased with increasing roll pressure accompanied with decreasing the tensile strength of produced tablets (Fig. 2). This result was in agreement with previous studies [2, 3]. It could be explained by the relationship between the density, the particle size of granules and the tensile strength of tablets. With increasing roll pressure the mean particle size of granules was increasing while the tensile strength of the final tablets was decreasing. This is suggested due to the decreasing bonding area with increasing particle size of granules.

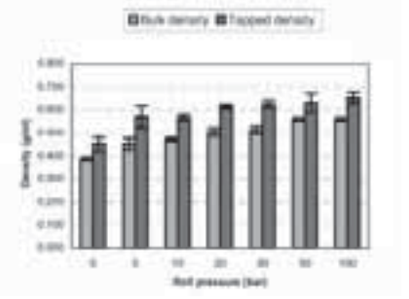

Figure 1: Bulk- and tapped density of granules prepared by roll compaction (Mean ± S.D., n=3)

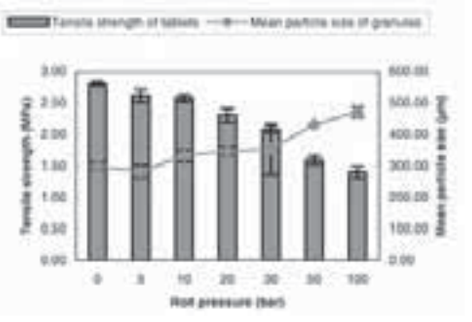

Figure 2: Mean particle size of granules and tensile strength of produced tablets

CONCLUSION

The roll pressure showed considerable influence on the properties of resulting granules and tablets. It is necessary to optimize process variables to obtain desirable product, therefore further investigations on the interactions with other process variables are in progress in our laboratory.

REFERENCES

1. Martinello T. *et al.* Optimization of poorly compactable drug tablets manufactured by direct compression using the mixture experimental design. Int. J. Pharm. 322: 87-95 (2006).
2. Parrot E.L. Densification of powders by concavo-convex roller compactor. J. Pharm. Sci. 70: 288-291 (1981).
3. Sun C.C., Himmelspach M.W. Reduced tabletability of roller compacted granules as a result of granule size enlargement. J. Pharm. Sci. 95: 200–206 (2006).

PO102

Comparison of the halving of tablets prepared by eccentric and rotary tablet press

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INTRODUCTION

The dividability of scored tablets is an important problem in the pharmaceutical industry, because the new guidelines require verification of the ease with which such tablets can be broken in half. The halving is a difficult problem, influenced by many parameters e.g. the properties of the materials, and the compression process. We have made tablets from binary powder mixtures by direct compression, a method that is more economical, than compressing with preliminary granulation. The results can be analyzed with using different mathematical equations (Walker, Heckel, Kawakita-Lüdde) or through artificial neural network modeling. It is a very useful optimizing method, because these systems can accommodate to different problems through change of their architecture. Our aim was to study the breaking properties of tablets prepared by different tablet machines, and compression forces.

METHODS AND MATERIALS

The tablets were prepared from binary mixtures of microcrystalline cellulose (Vivapur 102, J.Rettenmaier & Söhne, Germany) and mannitol (Pearlitol SD 200, Roquette Pharma, France), which were lubricated with 1% of magnesium stearate (Ph. Eur.).

The particle size distribution of powders was examined with a Laborlux S light microscope and a Quantimet 500 MC image analyzing system (Leica Cambridge Ltd., UK).

A scanning electron microscope (SEM) (Hitachi 2400 S, Japan) was used to study the morphological properties of crystals, and tablets.

A Polaron sputter apparatus (Greenhill, UK) was applied to induce electric conductivity on the surface of samples.

A PharmaTest PTG 1 (Pharma Test Apparatebau GmbH, Germany) powder rheological tester was used to determine the flow properties (flow time, angle of repose and bulk density) of materials and powder mixtures.

The compaction behaviour of powders was determined with an Engelsmann Stampfvolumeter (JRS Pharma, Germany).

The five different powder mixtures (Table1) were mixed with a Turbula mixer (Willy A. Bachofen Maschinenfabrik, Switzerland) (8+2 min, 50 rpm).

The powder mixtures were compressed on a Korsch EK0 eccentric (E. Korsch Maschinenfabrik, Germany) and on a Ronchi AM8S rotary (Officine Meccanice F.lli Ronchi, Milano, Italy) tablet machine mounted with strain gauges and a displacement transducer was applied to compress the powder mixtures with flat, single punches 8-mm in diameter with a bisecting line. The air temperature was 22-25 °C at 57-65% relative humidity. The tablet mass was 0.18 g.

Table 1: Powder mixtures

Sample name	Vivapur 102	Pearlitol SD 200	Magnesium stearate
1	90 g	10 g	1 g
2	70 g	30 g	1 g
3	50 g	50 g	1 g
4	30 g	70 g	1 g
5	10 g	90 g	1 g

The hardness of tablets was measured with a Heberlein tablet hardness tester (Heberlein & Co AG, Switzerland).

The breaking force required to break tablets in half, was determined with a laboratory-constructed tablet hardness tester.

The Artificial Neural Network modeling was made by Neural Network module of StatSoft Statistica 6.1 software (StatSoft Inc., Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

From the results can be considered, that the two materials have different properties. The Vivapur 102 has anisometric particles, and exhibits poor flow, and acceptable compaction properties, which can improved with adding Pearlitol SD 200. This powder has isometric particles and showed more better results in the preformulation studies. The physicochemical properties of the powder mixtures changed tendentiously between the two endpoints of the raw materials(Fig. 1). All powder mixtures were well compressible on both tablet machines, without the fluctuation of tablet mass.

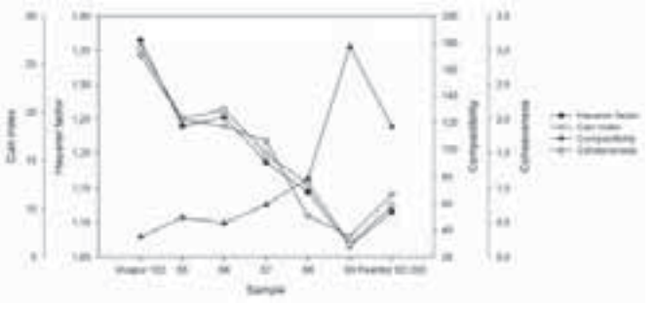

Figure 1: Flow and compaction properties of materils and mixtures

The results of the compression process were analyzed with artificial neural networks and with mathematical equations. The results of the two methods were similar, the response surfaces for the different parameters and the constants calculated by the equations displays an optimum point of the results by the 50% Pearlitol quantity. Above this the favourable properties of Vivapur 102 succeed not anymore, and the main parameters of tablets strongly spoils, because the very low cohesiveness of Pearlitol SD 200 results small interparticulate binding forces. In accordance to this, the tablet hardness and the tablet density decreases with increasing of Pearlitol quantity.

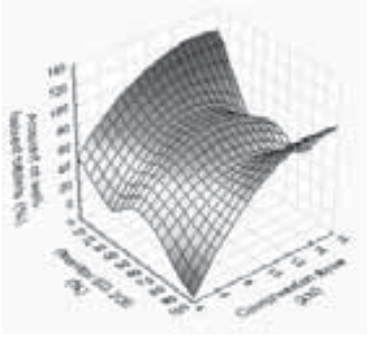

Figure 2: Response surface for the halving of tablets

This highly influences the breaking properties of the tablets, because tablets with low hardness often break into not equal halves, or their breaking surfaces crumble strongly. This problem is more significant by rotary tablet machines, because the these results lower tablet hardness by same compression force like the eccentric ones. The breaking properties are also influenced by the compression process, because tablets with uniform structure shows better breaking properties by lower hardness too. Advance of the artificial neural network model, that in contrast the mathematical equations (which describes only the compression process) can directly model the breaking of tablets (Fig. 2).

CONCLUSIONS

Preformulation tests on raw materials and powder mixtures showed that Pearlitol SD 200 can highly improve the poorer flow properties of Vivapur 102. The compression process can be described well with the equations of Heckel, Walker and Kawakita, but with artificial neural networks the breaking properties can be directly modeled.

ACKNOWLEDGEMENT

This work was supported by a Sanofi-Aventis scholarship.

References

Patel et al. Pharmaceutical Research 24 111-124 (2007)

PO103

Characterization of direct tablet compression agents analyzing compression force as a function of time

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INTRODUCTION

Tablet production depends on compression characteristics of the active pharmaceutical ingredient and the excipients where the densification process and the compaction behavior of the formulation requires characterization and optimization of powder the composition [1].

Direct compression is the most popular method in the manufacture of tablets because it is easier and quicker technique then wet-granulation. Various factors have been investigated and recognized as keystones for tablet production, e.g. flowability, highest particle pack, brittle fracture, plastic/elastic deformation. Lubricants are needed to reduce friction between the tablets and die wall during compaction and ejection [2].

During tablet formulation design, single punch eccentric tablet presses are often applied since they need small amount of powder as well as the tablet press instrumentation is not too expensive. Studies on compression profiles may serve as fingerprints and give the advantage in troubleshooting [3,4].

The compressibility can be characterized by experimental compression force as a function of displacement or time data. The force-time curve obtained can be described by shape parameters (skwewness and kurtosis) as well as deriving parameters such as effective force, proportional areas under the curve, time at maximum force [5]. The time dependence curves may emphasize the mechanism occurring during one cycle indicating the extent of elastic versus plastic response [6].
The objective was to characterize the compaction behavior of direct compression excipients analyzing primarily the force-time profile recorded by tablet press instrumentation. As commonly used lubricant, magnesium stearate was applied at different levels to investigate the effects on compaction as well as on tablet characteristics.

EXPERIMENTAL METHODS

Materials

The following materials were used as received from the suppliers: galenIQ™ 720 (BENEO-Palatinit GmbH, Mannheim, Germany) as agglomerated isomalt (Ph.Eur., USP/NF); microcrystalline cellulose (MCC)

as Vivapur 102 contain (Rettenmaier & Söhne, Rosenberg, Germany); magnesium stearate as lubricant (REANAL, Budapest, Hungary).

Tablet compression

All of the powder blends were made utilizing a cubic mixer (Type UG, Erweka Apparatebau GmbH, Heusenstamm, Germany). The lubricant magnesium stearate was added to each of the direct compression agents at 0.5%, 1% and 2% level. The mixing time was 10 minutes at 20 rpm.

Tablets were compressed using a single punch eccentric tablet press (Type TM 20, Diaf, Copenhagen, Denmark) with flat, sharp-edged punches of 10 mm diameter at 40 tablets per minute speed. Three different compression forces (10 kN, 15 kN, 20kN) were applied. Tablet mass was kept in the range between 300 to 320 mg at ambient conditions.

Tablet press instrumentation

The instrumentation involved strain gauges (KMT-LIAS-06-3/350-5E type, Kaliber Ltd., Budapest, Hungary) on both the upper and lower punches. The displacement was measured by a magnetic sensor (Limes L2 type, Kübler GmbH, Villingen-Schwenningen, Germany) with a resolution of 5 mm and signaling at 1 μs pulse interval. All signs were transferred through cables to the data recorder (USB-6210, National Instruments Corp., Austin, USA) involving signal conditioning. The data acquisition was done using NI-DAQmx 8.3. software (National Instruments Corp., Austin, USA). The calibration was made by Kaliber Ltd. Budapest, Hungary. 1000 tablets were prepared for each batch.

4000 data points were measured during a single compression cycle which were analyzed by MS Excel macroprograms written in-house. The mathematical processing involved normalization and estimation of parameters e.g. effective force, areas under the compression force-time curve [5, 6].

Tablet hardness

The radial breaking strength of the tablets was measured with a hardness tester (TBH 200, Erweka instrument GmbH Heusenstamm, Germany).

RESULTS AND DISCUSSION

The time dependence curve area divided into partial areas can be associated with the compaction and decompaction stages (Figure 1).

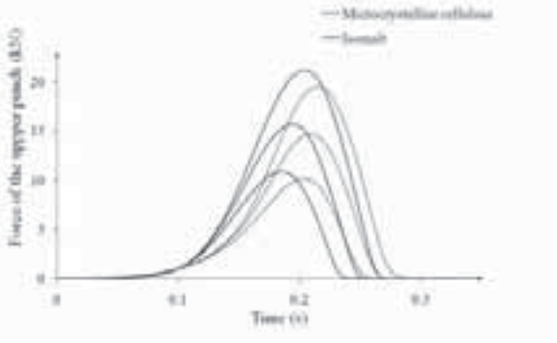

Figure 1: Upper punch compression force as a function of time at 1% magnesium stearate level

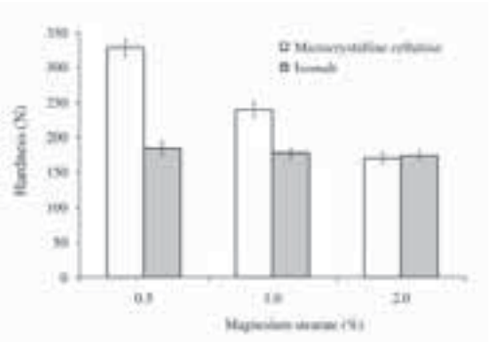

Figure 2: The impact of lubricant amount on the tablet hardness

Both microcrystalline cellulose and isomalt based direct compression excipients demonstrated similar force-time profiles at 1% magnesium stearate level.

Although tablets prepared with microcrystalline cellulose had higher hardness at 0.5% of magnesium stearate, no significant difference was observed at higher (1-2%) magnesium stearate level.

CONCLUSION

The force-time curves are useful indicators of the compaction behavior regarding elasticity and plasticity, microcrystalline cellulose and isomalt based excipients demonstrated similar force-time profiles at 1% magnesium stearate level. The results suggest that magnesium stearate for both direct compression excipients.

REFERENCES

1. Busignies, V., Leclerc, B., Porion, P., Evesque, P., Couarraze, G., Tchoreloff, P. Compaction behaviour and new predictive approach to the compressibility of binary mixtures of pharmaceutical excipients. Eur J Pharm Biopharm. 64: 66-74. (2006)
2. Zuurman, K., Van der Voort Maarschalk, K., Bolhuis, G.K. Effect of magnesium stearate on bonding and porosity expansion of tablets produced from materials with different consolidation properties. Int. J. Pharm., 179: 107-115 (1999)
3. Hoblitzell, J.R.; Rhodes, C.T. Determination of a relationship between force-displacement and force-time compression curves. Drug Dev. Ind. Pharm. 16: 201–229 (1990)
4. Antikainen, O., Yliruusi, J. Determining the compression behaviour of pharmaceutical powders from the force-distance compression profile. Int J Pharm. 252: 253-261 (2003).
5. Juppo, A.M.; Kervinen, L.; Yliruusi, J. Skewness and kurtosis of force-time profile obtained from compression of lactose, glucose and mannitol granules. Eur. J. Pharm. Biopharm. 41: 374-381 (1995).
6. Yliruusi J.K.., Antikainen O. K. New parameters derived from tablet compression curves. Part I. force-time curve. Drug Dev. Ind. Pharm. 23:, 69-79 (1997)

PO104

Investigation on the parameters influencing subdivision of scored tablets

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INTRODUCTION

Tablets with single or multiple score line allow the administration of a proportion of the tablet which can be broken without compromising drug effectiveness or drug safety in many cases [1, 2]. The advantages of breaking scored tablets can be summarized as follows [3]:

- ease of swallowing,
- dose flexibility in geriatrics and pediatrics,
- dose flexibility for dosing on need,
- dose flexibility on increasing and decreasing dosage schedules,
- cost reduction.

However, only few studies deal with the factors influencing the breakability of tablets [4]. Disfunctions of score lines were reported for some cases, where many scored tablets demonstrated unsatisfactory mass uniformity of the subdivided halves. From the patient's perspective, the score lines are important for self-decided dosing and ease of swallowing. This data suggested that improving the functioning of score lines may be a more practical approach than banning this dosage form [5]. It is now clearly recognized that performing score line is a critical parameter in the design of quality. The European Pharmacopoeia [6] includes a test on the subdivision of tablets.

During the tablet compression process complex movements take place within the powder bed and interactions occur between the powder and tooling of the tablet press such as die wall and punch faces. It should be noted that density variations influence the physical and mechanical properties of the compact [7].

The aim of this study was to investigate the influence of various tablet parameters (e.g. shape, size, deepness of score line, etc.) on the secability. Force displacement measurement of the scored tabletting punches and different compression forces were used to compare the secability of the tablets.

MATERIALS AND METHODS

The tablets applied for this study were manufactured by direct compression using an instrumented Fette single punch tablet machine type EXI. For the best comparison of the secability data the same placebo composition was used in the experiment: Microcrystalline Cellulose (Ph.Eur.); Lactose Monohydrate (Ph.Eur.); Anhydrous Colloidal Silicon Dioxide (Ph.Eur.); Magnesium Stearate (Ph.Eur.). During the tabletting process three different compression forces were used on different tablet mass and shape (round convex, round flat and oblong) using different shape and deepness of dividing line.

During compression, the press force of the upper and lower punch and the displacement of the upper punch were registered in 1000 measuring points in the case of 10 tablets, respectively. A Microsoft Excel macro language program was used for the data processing and analysis.

During the tablet halving experiment the tablets were broken by hand with dividing line facing upwards. The mass of each tablet half unit was measured by Mettler AT 400 analytical balance with resolution of 0.1 mg. In order to compare 30 scaling point the data were characterized with AV value (acceptance value):

$$AV = |M - X| + ks.$$

The maximum allowed acceptance value in the case of secability was 15.

The mass and the resistance to crushing of the tablets were measured by Pharmatest tablet hardness tester type WHT-1, friability of tablets was measured by Pharmatest friability tester type PTE 20E, disintegration time of tablets was measured by Erweka disintegration tester type ZT4.

RESULTS AND DISCUSSION

According to our results the tablet mass (thickness) and the applied press force has a significant influence on the secability of the tablets prepared with the same tabletting tools. The secability of the small size round convex tablets improve with the higher press force in case of low tablet mass. On contrary by increasing the tablet mass the secability of the tablets fails with higher pressing force.

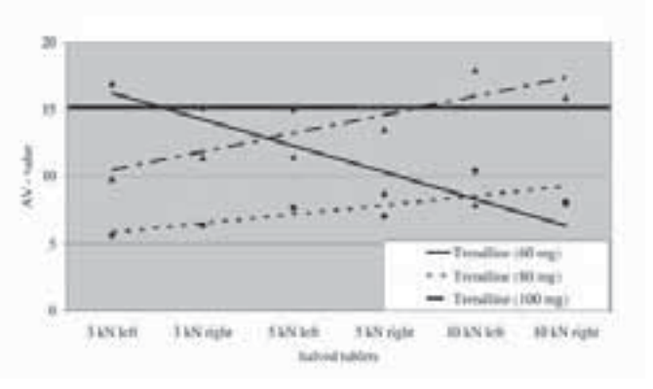

Figure 1: The effect of the tablet mass and press force on the secability of 6 mm round standard convex tablet

The secability of the tablets improved with changing the tabletting punches from round shape to oblong shape.

On the basis of our results the shape is the significant factor, oblong shaped tablet form can resist to the impact of the tablet mass variation. The influence of the score line on the secability of an oblong flat tablet shape indicated that tablets without breaking mark have no appropriate secability quality. Using break mark on one side of the tablets, moreover using break marks on either side of the tablets increase significantly the breaking accuracy of the tablets.

CONCLUSION

This experimental work illustrates that the secability of the tablets can be influenced by wide range of parameters like tablet size, shape, mass, thickness, compaction force, curvature of the tablets as well as the deepness and form of the score line on the tablet. It was observed, that density variations of the powder after compaction influenced the physical and mechanical properties of the tablets. The high density region of the tablets under the score line may cause unequal proportion of divided tablets.

References

1. Duncan, M.C., Castle, S.S., Streetman, D.S., 2002. Effect of tablet splitting on serum cholesterol concentrations. Ann. Pharmacother. 36 (2), 205-209.
2. Peek, B.T., Al-Achi, A., Coombs, S.J., 2002. Accuracy of tablet splitting by elderly patients. JAMA 288 (4), 451–452.
3. van Santen, E., Barends, D.M., Frijlink, H.W., 2002. Breaking of scored tablets: a review Eur. J. Pharm. Biopharm. 53, 139–143.
4. Rodenhuis, N., De Smet, P.A.G.M., Barends, D.M., 2003. Patient experiences with the performance of tablet score lines needed for dosing. Pharm. World Sci. 25 (4), 173–176.
5. Rodenhuis N, De Smet PA, Barends DM. The rationale of scored tablets as dosage form. Eur J Pharm Sci. 21: 305-308. (2004)
6. Europen Pharmacopoeia 6th Edition, 2008.
7. Sinka I.,. Burch S.,.Tweed J., Cunningham J.C. Measurement of density variations in tablets using X-ray computed tomography Int. J. Pharmaceut. 271: 215-224 (2004)

PO105

An approach to modifying the release of acetaminophen tablets using simple technologies

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INTRODUCTION

Acetaminophen (AAP, sin. paracetamol) is a widely used analgesic and antipyretic drug which is quickly metabolized in the body ($t_{1/2}$ = 2-3 hours). It's hydroxylated metabolite is hepatotoxic in high concentration [1]. Because of this characteristic and also in order to improve patient compliance (especially in children), studies have been made in order to control AAP release from the pharmaceutical form. One of the succesfull aproaches was to coat AAP particles with an acrylic polimer, using fluid bed coating technology. [2]

The aim of this study was to obtain similar-acting formulations, but using low-tech methods, which often are more usefull for the generics manufacturer, due both to price and equipment availability.

EXPERIMENTAL METHODS

Materials. Methods.

For this purpose, we used wet-granulation technology. Four formulas were tested, formulation percentages were calculated in order to yield granules containing 80% AAP, as follows:

Uncoated AAP granules were obtained by wet granulation through a 0.8 mm mesh sieve, drying at 30 °C and homogenization through a 1.25 mm mesh sieve. The binder used was a mix of 20% corn dispersion and 9% gelatine solution.

Table 1: Granules formulation:

Formulation	I	II	III	IV
Substance	%	%	%	%
AAP	80	80	80	80
Eudragit E 30 D	0	0.08	0.48	0.8
Lactose 200 mesh	10.707	10.627	10.227	9.907
Corn starch	8.141	8.141	8.141	8.141
Gelatine	1.151	1.151	1.151	1.151
Total	100	100	100	100

For formulas II, III and IV, Eudragit E 30 D was applied to dried uncoated granules as a 0.5%, 3% and respectively 5% dispersion. The coating was done using a Caleva Mini Coater/Drier 2 with a modified sieve. Sieve modification was necessary in order to prevent material loss.

After final powdering and mixing, the uncoated material was compressed using an in-house instrumented eccentric Korsch-type tabletting machine tooled with 12mm punches, in order to obtain 550mg

tablets. Seven pressure ranges, from 20 to 175 MPa, were used. After compression, the tablets were tested for hardness (Vanderkamp VK200 tablet hardness tester), friability (Vankel friabilator) and dissolution profile (USP XXX, App 2, 50 RPM, pH 5.8 [III]). An optimum pressure range was established for this formulation.

Table 2: granule powdering ratio

Material	%
Acetaminophen granules (80% AAP)	90
Corn starch	6.48
Magnesium stearate	1
Talcum	2.52
Total	100

The three coated batches were also powdered and mixed and then compressed at about the optimum pressure range (40 MPa). The tablets were tested for the same parameters.

RESULTS

Formulation I (uncoated AAP)

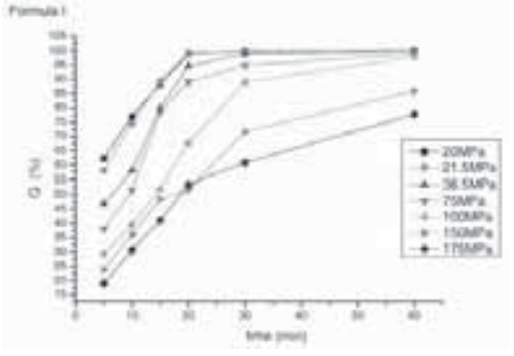

Figure 1: In vitro release profile of Formulation I compressed at seven pressure ranges

Formulations II, III, IV (coated AAP)

Friability and hardness were good for all three coated formulations, being quite similar with the first formulation. However, the dissolution profiles are markedly different. The plot shows the also dissolution of the first formula at the same tableting pressure, for easier comparison.

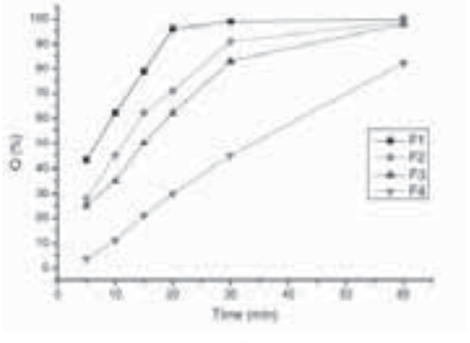

Figure 2: In vitro release profile for Formulation I, II, III and IV, compressed at 40 MPa

DISCUSSIONS

Uncoated material (Formulation I)

The uncoated material was compressed at seven pressure values, between 20 and 175 MPa. This study yielded two valuable informations:

- the optimum pressure range for this formulation (40-75 MPa)
- over 100 MPa, the uncoated AAP s dissolution rate tends to linearioty, with a slower and slower release rate.

However, the dissolution profiles are not linear, and the higher pressures (and therefore forces) used will lead to premature wear and tear of the tableting machine.

Coated material (Formulation II, III, IV)

As expected, coating does indeed modify the release profile. The concentration of the coating polymer correlates with the decrease of dissolution rate. It will be also noted that although the modification of the release profile is less marked than in the case of directly covering the AAP particles with the coating polymer[2], the dissolution profile is modified enough for the aimed purpose.

CONCLUSIONS

We have managed to modify the release of acetaminophen using two lower tech methods: tableting pressure modification and granule coating. The best dissolution is for the granules coated with 0.8% Eudragit E 30 D (5% aqueous dispersion).

References

1. Seong H.J, Min H.L., Myung G.L., Pharmacokinetics of acetaminophen after intravenous and oral administration to spontaneously hypertensive rats and normotensive Wistar rats , Pharm. Sci., 83, 810-814, 1994.
2. Comoglu T., Aydinli A., Baykara T., Determination of compresibility and in vitro release properties of acetaminophen granules coated with Eudragit E 30 D , Turk J. Med. Sci, 35, 333-335, 2005
3. The United States Pharmacopoeia XXX, United States Pharmacopoeial convention, 2006

PO106

Researches on the dissolution characteristics of alprazolam tablets

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INTRODUCTION

Currently, alprazolam is used worldwide for its tranquilizing and mild antidepressive actions. The pharmaceutical industry is producing alprazolam tablets using various formulations and manufacturing process techniques.

For the present study, we have prepared tablets with 0.25, 0.5 and 1.00 mgs of alprazolam, by direct compression. The active was associated in the formulation with excipients adequate for direct compression: Ludipress, Kollidon VA 64, Kollidon CL (BASF / Germany) and magnesium stearate (Peter Greven / Netherlands). The resulting tablets were then subjected to quality control tests.

We have studied the mechanical resistance and friability of the tablets, as well as the dissolution tests, monitoring the variation in time of the dissolution speed and dispersion of the tablets, and some stability studies for the next 6 months after the manufacture of the tablets.

The aim was to produce alprazolam tablets with a suitable mechanical resistance, minimal friability, and a short disintegration time and high dissolution rate.

EXPERIMENTAL METHODS

The formulations for the prepared tablets are shown in table I.

Table 1: The formulations for the 0.25 mg, 0.5 mg and 1 mg alprazolam tablets

Substan-ces	Quantity mg/tablets			Function in formulation
Alprazo-lam	0.25	0,.0	1.00	Active ingredient
Ludipre-ss	121.75	142.50	154.00	Filler, binder, disintegrant and flow improver
Kollido-n VA 64	3.00	4.00	5.00	Dry binder, disintegrant
Kollido-n CL	2.00	2.00	2.00	(Super) Disintegrant
Magnesium stearate	1.00	1.00	2.00	Lubricant
TOTAL	128	150	164	

Ludipress is custom-produced granules consisting of lactose monohzdrate, Kollidon 30, and Kollidon CL for use as an all-purpose direct compression excipient that fulfills the functions of filler, binder, disintegrant, and flow improver. [1]

Based on these theoretical considerations, we have prepared the tablets by direct compression.

All three formulations have lead to white round tablets, with a lenticullar, smooth and uniform surface, with a diameter of 8 mm.

The experimental results of the determinations performed on the tablets are listed in table II.

Table 2: The experimental results of the determinations performed on 0.25 mg, 0.5 mg and 1 mg alprazolam tablets

Tested parameters	0.25 mg Alprazolam tablets	0.5 mg Alprazolam tablets	1 mg Alprzolam tablets
Average weight, mg	128.12	149.86	163.25
Disintegration time, min.	2.6	2.9	3.0
Alprazolam assay, mg/tablet	0.243	0.498	0.987
Friability, %	0.3	0.2	0.4
Mechanical resistance, N	85	87	90

The experimental data obtained during and after the dissolution test are shown in the following table and figures. Table III shows the concentration of the solution containing the released active from the 0.25 mg tablets. [2,3]

Table 3: The concentration in alprazolam released from the six of 0.25 mg alprazolam tablets tested, at various moments in time

Parameter	Time (minutes)				
	5	10	15	20	30
Concentration%	70.21	87.89	92.16	94.70	98.30
Concentration%	69.72	86.82	92.90	95.15	98.87
Concentration%	70.45	87.45	92.26	95.73	98.22
Concentration%	70.11	86.98	92.54	95.81	98.63
Concentration%	70.36	87.13	92.31	96.06	98.78
Concentration%	69.32	86.76	92.08	95.96	99.09
Average value	70.0283	87.1717	92.3750	95.5683	98.6483
Dispersion	0.4299	0.4301	0.3012	0.5308	0.3367
Disp./Average value	0.0061	0.0049	0.0033	0.0056	0.0034

Figure 2 shows the overlapping of the variation in time of the concentrations for the 0.25 mg alprazolam tablets. The 3D image is quite eloquent regarding the variation pattern. The same pattern can be observed for the initial and final values. After the first 5 minutes, the amount of alprazolam released fluctuates from 69.32 % to 70.45 %, and after 30 minutes it exceedes 98 %. The variation is nonlinear, and can be considered as being of an exponential type. This assumption is supported by the calculated values for the variation of the average concentrations and of the average dispersion (figures 3 and 4).

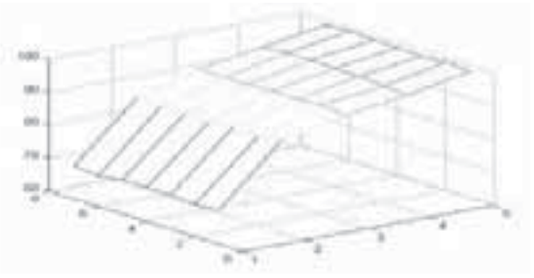

Figure 2: The variation of the concentration for the 0.25 mg alprazolam tablets

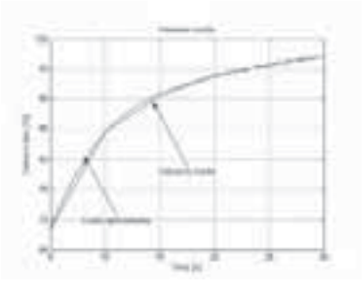

Figure 3: The variation of the average concentration for the 0.25 mg alprazolam tablets

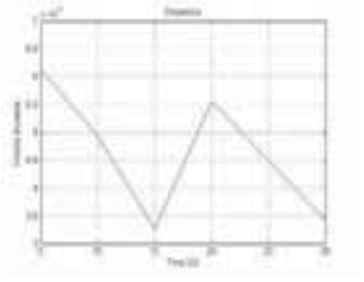

Figure 4: The variation of the dispersion for the 0.25 mg alprazolam tablets

The variation in time of the alprazolam concentration for the 6 0.50 mg alprazolam tablets is similar, with only slight changes from one tablet to another. The charts shown in figures 6 and 7 are suggestive for the variation in time of the average concentration and the dispersion for the alprazolam tablets. The variation of the concentration in time is allmost identical for each tablet.

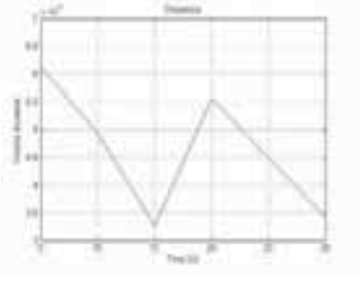

Figure 6: The variation of the average concentration for the 0.50 mg alprazolam tablets

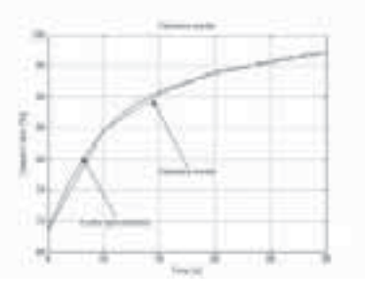

Figure 7: The variation of the dispersion for the 0.50 mg alprazolam tablets

The same tests also with very good results were made on 1 mg alprazolam tablets.

After these determinations, it can be observed that the concentration values are within the limits specified by USP provisions, over 75 % of the tablet being dissolved after 30 minutes, for both types of metoprolol tablets.

The stability of the prepared tablets was studied for six months.

CONCLUSIONS

The results obtained in the quality and quantitative determinations performed on the tablets show that the determined characteristics are optimal and within the limits provided by current standards, for the three types of prepared tablets. They all show a good mechanical resistance and a very low friability and excellent disintegration intervals and dissolution rates.The dissolution curve does not change its aspect significantly with the variation of the concentration.

The tablets were stable during storage and handling over the six months. The chatacteristics showed only insignificant variations from the initial values.The results show the reproductibility of the data and the stability of the final product, this type of tablets being suitable for use in therapy.

References

1. Gohel M.C., Pranav D.Jogani, A review of co-processed directly compressible excipients, Pharmaceutical Science 8 (1), 76-93, (2005).
2. Ansel C.H.ºi colab. Pharmaceutical Dosage Form and Drugs Delivery Systems, Ed.Williams & Wilkins, 1995;
3. Moore J.W., Flanner H.H., Mathematical comparison of curves with an emphasis on vitro dissolution profiles, Pharm. Tech. 1996;20:64-74.

PO107

Effect of different types of paracetamol on tablet characteristics and dissolution rates

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INTRODUCTION

Paracetamol, also known as acetaminophen, has a long history in clinical use as an effective antipyretic and analgesic, but it is surprising that the precise mechanism of action of paracetamol is still not known. On the other hand, paracetamol powder exhibits poor compressibility, low flowability and its tablets show a capping tendency. Poor compaction behavior of paracetamol and its elastic deformation has been connected with polymorphic forms of paracetamol. Even though they are chemically identical, the different polymorphic forms show different free energies, and, therefore, have different physical properties that can influence significantly the product performance. These include differences in solubility and dissolution rate (affecting bioavailability), solid-state stability (affecting potency), deformation characteristics (affecting compressibility), and particle size and shape (affecting powder density and flow properties). The main problem in manufacturing of tablets with paracetamol is the choice of method, which is most appropriate for industrial manufacturing of tablets. There are several methods for modification of crystalline structure of paracetamol, in order to manufacture tablets with direct compression. Examples include spherical crystallization, crystallization from different solvents to produce different crystal habit, incorporation of additives by co-precipitation and development of sintered-like crystals. The aim of this study was to produce immediate release tablets with direct compression and to investigate the effects of different types of paracetamol on tablet characteristics and dissolution rates.

MATERIALS AND METHODS

Paracetamol (monoclinic form, Huzhou Konch Pharmaceuticals Co. Ltd.), Paracetamol D.C. (Mallinckrodt inc. St.Louis), Paracetamol coated (Ethypharm SA, France) were chosen as an active ingredient; Kolidon® VA 64 (BASF, Germany) and Klucel®-EXF (Aqualon, Germany) were used as binders; Polyplasdone® XL-10 (ISP, Switzerland) and Vivasol® (JRS Pharma) were used as superdisintegrants; Avicel® PH 200 (supplied by Lek-Sandoz) was used as a filler, Mg-stearate (Pharmachemic, Belgium) and Aerosil® 200 (Degussa, Germany) were used as a glidants. For each of components and final mixture used for preparation of tablets it have been determined the particle size distribution (sieve analysis, apparatus Retsch), bulk density, tapped density (Vankel Apparatus), true density (Accupyc 1330), and contact angle (Kruss DSA 100). Flow rate was measured using a standard funnel as described in Ph. Eur. V. The scanning electron microscope was using to observe the morphology of the acetaminophen powders.

Preparation of tablet

Tablets of 700 mg in weight and 12 mm in diameter were prepared by direct compression using single-punch tableting machine, Kilian SP 300.

Table 1: Composition of tablets mixture (First combination has Vivasol as a superdisintergrant (5%) and second combination has a Polyplasdone XL-10 in concentration of 4% as a superdisintergrant)*

COMPOSITION	CONTENT [%]
Paracetamol (crystalline or D.C or coated)	75
Kolidon VA 64	4
Vivasol/Polyplasdone XL 10*	5 (4)*
Avicel PH 200	11.5 (12.5)*
Klucel EXF	2
PEG 4000	1
Mg-stearate	1
Aerosil ® 200	0.5

The following assays have been performed: organoleptic control, uniformity of mass, height, hardness (Tablet hardness tester, Vanderkamp VK 200), friability (Erweka tar 10) and disintegration time (Erweka apparatus type ZT4 -1). Dissolution profiles were measured, for the tablets according to the USP XXIII paddle method at 50 rpm/min. Dissolution rates were determined in buffer solutions pH=1.0 (0.1 N HCl; pH=4.5 (phosphate buffer), pH=5.8 (phosphate buffer), pH=6.8 (phosphate buffer).

RESULTS

The whole process of direct compression has been described in terms of compressibility, elastic recovery, compactibility, disintegration, and drug release. The paracetamol powder flowability was determined by the flow rate, angle of repose, and percentage of compressibility (Table 2).

Table 2: Experimental values of compressibility, flow rate and angle of repose of different paracetamol powders

Flow properties	Paracetamol crystalline	Paracetamol D.C.	Paracetamol coated
Carr s index	47.47 %	28.11%	12.36 %
Hausner ratio	1.903	1.393	1.14
Flowability	59,9 s	35 s	30 s
Angle of repose	∞	41.63 ˚	39.52 ˚

First of all, different physical properties of paracetamol powders are connected with particle size and shape. Needle shape crystals of monoclinic form of paracetamol and very high surface to mass ratio result in extremely poor flowability and compression characteristic. Furthermore, mean particle size of paracetamol D.C. is 45 μm and consequently powders become more cohesive and flow problems are evident. On the other hand, particles shape of coated paracetamol is more spherical with minimal interparticle contacts, what provide optimal flow properties. Because of the high content of paracetamol in mixture, physical characteristics of present paracetamol will have essential influence on the compression properties and elastic behavior of the tablet powder mixture. It could be explained with dominant paracetamol-paracetamol interactions, which provide more cohesive properties of mixture and consequently poor flowability. Also, these contacts have an influence on elastic recovery, which has been associated with the storage of elastic energy during compression as deformation energy under stress and subsequent release of this energy after removal of axial pressure. It was impossible to make tablets with lactose superdisintegrants (Starlac, Microcellac, Ludipress) because spay-dried lactose in this formulation has limited ability to form strong tablets and it has low dilution potential, so it is primarily used in tablets in which it forms the major ingredient. It was impossible to compress tablet mixture with untreated paracetamol with automatic method on single punch tableting machine because of very high elastic relaxation and capping of the prepared tablets. To improve compression properties we used modified paracetamol (Paracetamol for D.C. and coated Paracetamol). These powders are mixtures with binding agents such as gelatin, PVP, starch and starch derivates. For increasing tablet strength and reduction in tablet friability and capping, the inherent mechanical properties of the tablet binder providing greater toughness and plasticity are important, in addition to specific surface area and particle size. Hydroxypropylcellulose (Klucel® EXF) has excellent thermoplastic characteristics and has a high degree of plastic flow. It has been confirmed that in concentration of 2% in formulation we overcome capping. Tablets containing modified paracetamol have appropriate friability (0.5%) and disintegration time. On the other hand, it is evident that the type of a superdisintegrants has a high influence on dissolution rate. Sodium carboxymethylcellulose provides better dissolution rate than crosspovidone. Tablets with coated paracetamol have grater porosity and better dissolution rates in comparison to paracetamol D.C.

CONCLUSION

D.C and coated paracetamol are appropriate for producing tablets by direct compression when more than 40% of paracetamol is included. Compression forces are much smaller than in case of paracetamol monocrystalline form. However, friability of tablets is still appropriate. Percentage of dissolved paracetamol after 30 minutes is more than 85% in all investigating buffer solutions what confirms the used approach in preparing the tablets with immediate release.

REFERENCES

1. M. Tiago K., Telma Mary,V. R. Velasco S.Taqueda. Optimization of poorly compactable drug tablets manufactured by direct compression using the mixture experimental design, Int J Pharm. 322 (2006) 87-95.
2. H. Mohammed, B.J. Briscoe, K.G. Pitt. A study on the coherence of compacted binary composites of microcrystalline cellulose and paracetamol, Eur J Pharm Biopharm. 63 (2006) 19–25.
3. H. A. Garekani, J. L. Ford, M. H. Rubinstein, A. R. Rajabi-Siahboomi. Highly compressible paracetamol - II. Compression properties, Int J Pharm. 208 (2000) 101-110.

PO108

Stable pharmaceutical formulation of incompatible active ingredients

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INTRODUCTION

Stability is one of the most important factors which determines whether a compound or a mixture of compounds can be developed into a therapeutically useful pharmaceutical product. Impurity control is a continuing concern of regulatory agencies and the pharmaceutical industry. Impurities must be controlled because of their potential toxicity. However, impurities that are formed by degradation of the active ingredient during manufacturing of dosage forms or resulting from storage are common impurities in the medicines. Currently valid guidelines on impurities in active substances and dosage forms require characterization of recurring impurities and degradation products found at or above an apparent level, depending on maximum daily dose. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products.

Therefore, it is essential, to maintain proper chemical stability of the active substance in dosage form during stability testing and at normal storage conditions.

When mixing different ingredients in a pharmaceutical formulation there exists the possibility of interactions taking place between the components. In addition, each component may have different degradation characteristics. This can subsequently lead to problems arising in the chemical stability of the active ingredient in the final dosage formulation.

Telmisartan or its alkaline salts may be used as an angiotenzin II antagonist. Telmisartan itself is a poorly soluble substance, and in order to increase its bioavailability, it is normally administered as an alkaline salt, such as sodium salt or incorporated into a composition which itself provides for an alkaline pH. It is advantageous to administer it together with hydrochlorothiazide or to manufacture a composition comprising both to treat hypertension. Such composition requires a specific technology (bilayer tablet) in order to prevent the degradation of hydrochlorothiazide which occurs when exposed to alkaline media. Therefore a process for the manufacturing of a stable combination of telmisartan and hydrochlorothiazide using conventional equipment is

needed overcoming the adverse effect on the stability of hydrochlorothiazide caused by alkaline active ingredient or inactive ingredients in the pharmaceutical composition.

EXPERIMENTAL

In a search of alternative technologies we observed that the chemical stability of hydrochlorothiazide in a combination formulation with telmisartan can be significantly improved by means of a coated composition comprising telmisartan in the core and a hydrochlorothiazide in the coating, wherein the hydrochlorothiazide layer could be applied as film coating or sugar coating. The interactions between hydrochlorothiazide layer and telmisartan core can be additionally improved by a separating coating and/or by one or more acidic component or a combination of acidic components, characterized in that a 1% (w/v) aqueous dispersion has a pH < 6, are incorporated in the hydrochlorothiazide part of the combination formulation. Optionally, acidic components can also be incorporated in the separating coating.

We were able to manufacture a stable composition comprising telmisartan and hydrochlorothiazide by first preparing tablet cores comprising of telmisartan, binder, solubile diluent and alkalizing agent. Between the outer layer (comprising of hydrochlorothiazide, colloidal silicon dioxide, PVP, plasticizer and optionally acidifying agent) and tablet core, a separating layer comprising of any polymer appropriate for conventional film coating (like cellulose ethers, acrylic polymers, PVPs, PEGs) can be applied.

Example 2

TABLET CORE WITH TELMISARTANE	
COATING WITH HCT	
HYDROCHLOROTHIAZIDE	active pharm. ingredient
COLLOIDAL SILICONE DIOXIDE	emulsion stabilizer, acidifying agent, film disintegrating agent
CITRIC ACID	acidifying agent
POVIDONE	film/matrix forming polymer, HCT stabilizer
ETHANOL 96%	solvent
DEMI WATER	solvent

Example 3

TABLET CORE WITH TELMISARTANE	
INTERMEDIATE COATING	
POVIDONE	film forming polymer
ETHANOL 96%	solvent
DEMI WATER	solvent
COATING WITH HCT	
HYDROCHLOROTHIAZIDE	active pharm. ingredient
COLLOIDAL SILICONE DIOXIDE	emulsion stabilizer, acidifying agent, film disintegrating agent
CITRIC ACID	acidifying agent
POVIDONE	film/matrix forming polymer, HCT stabilizer
ETHANOL 96%	solvent
DEMI WATER	solvent

We have detected the degradation products by HPLC using following procedure: 50 l samples were at 30 °C eluted on Hypersil BDS C18, 5 m, 250 X 4.6 mm column at flow rate 1.5 mL / min using following gradient:

t (min)	0	10	25	45	50	51
% A	90	90	50	20	20	90

A = Solution of KH_2PO_4 / Et_3N with pH 3.5,
B = Solution of KH_2PO_4 / Et_3N with pH 3.5 and acetonitrile 1 : 4
and detected by UV at λ = 228 nm.

RESULTS AND DISCUSSION

We have prepared pharmaceutical compositions comprising telmisartan and hydrochlorothiazide (example 2 and 3), exposed them to stress stability testing at 60 °C for 14 days and compared the amount of formed degradation products to the samples of commercially available bilayer tablets comprising telmisartan and hydrochlorothiazide (reference). We have on one hand followed the amount of all impurities as well as specifically the amount of DSA (the main degradation product of hydrochlorotiazide).

Results are presented in following table:

Example no	Testing condition	Sum of impurities	DSA
1- dry mixture of active ingredients	0	0.19	0.06
	14 days, 60 °C	4.05	2.99
2	0	0.16	0.09
	14 days, 60 °C	0.85	0.73
3	0	0.18	0.11
	14 days, 60 °C	0.35	0.28
Reference	0	0.61	0.08
	14 days, 60 °C	0.86	0.36

The results show that:

- the presence of polyvinylpyrrolidone and colloidal silicon dioxide in outer layer of tablet with hydrochlorothiazide (core containing telmisartan), reduces the degradation of hydrochlorothiazide in pharmaceutical composition (***Example 2***)
- hydrochlorothiazide substance can be additionally stabilized by the presence of an acidic ingredient such as citric acid and an intermediate layer (***Example 3***)

CONCLUSION

For bilayer tablets manufacturing a special tabletting machinery is required, therefore a process for the manufacturing of a stable combination of telmisartan and hydrochlorothiazide using conventional equipment is needed overcoming the adverse effect on the stability of hydrochlorothiazide caused by alkaline active ingredient or inactive ingredients in the pharmaceutical composition.

In a search of alternative technologies we observed that the chemical stability of hydrochlorothiazide in a combination formulation with telmisartan can be significantly improved by means of a coated composition comprising telmisartan in the core and a hydrochlorothiazide in the coat-

ing, wherein the hydrochlorothiazide layer could be applied as film coating or sugar coating. The interactions between hydrochlorothiazide layer and telmisartan core can be additionally improved by a separating coating and addition of one or more acidic components.

Reference

1. WO 03/059327 – Bilayer pharmaceutical tablet comprising telmisartan and a diuretic and preparation thereof. T. Friedl, G. Schepky (2003).
2. WO 200714475 – Pharmaceutical composition. A. Beso, I. Legen, S. Reven (2007).

PO148

A preliminary study of the effect of an aqueous enteric film coat deposition on the film coat structure and dissolution properties of model drug tablets

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OBJECTIVES

To study the effects of coating solids content, and quantity of coating applied, on the performance of enteric-coated tablets coated with an aqueous acrylic coating, and to assess the suitability of using Terahertz imaging technology, employed off-line, to evaluate coating uniformity, and coating structure at the interface between both the tablet core and a sub coating and between the sub coating and the enteric coating.

EQUIPMENT

- Piccola tablet press (Riva)
- Disintegration equipment (PharmaTest)
- Dissolution Equipment (PharmaTest)
- Dissolution – UV spectrophotometer (Cecil 3021)
- Friabilator (PharmaTest PTF E)
- Tablet hardness tester (PharmaTest PTB)
- Labcoat IIX (O'Hara) fitted with a single Schlick spray gun with the ABC cap.

METHODOLOGY

Compression Details

Ingredient	mg/tablet	%
Theophyline anhydrous	25.0	6.25
Lactose monohydrate	263.0	65.75
Microcrystalline celulose	80.0	20.0
Copovidone	20.0	5.0
Crospovidone	8.0	2.0
Colloidol silicon dioxide	2.0	0.5
Magnesium stearate	2.0	0.5
Total tablet weight	400.0 mg	

Theophylline Tablet Formulation

Tablet Press – Riva Piccoia 8 station	
Weight	400 mg ± 3%
Diameter	11 mm 0,5%
Punches	Round Normal Concave (50% plain/ 50% engraved ADVANTIA/ISP)
Breaking Force	11 kp
Friability	0.04%
Disitegration	1' 45"

The tablets were coated using Advantia Prime 144900BA01 (yellow) to a 3% weight gain and subsequently with Advantia Performance 190024HA49 (white) enteric coat.

COATING DESCRIPTION

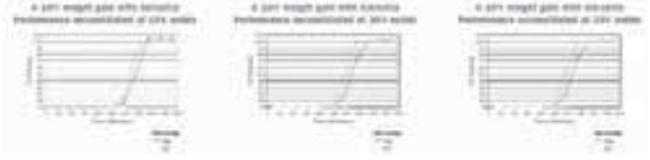
Coating was carried out in an O'Hara Labcoat IIX fitted with the 19 inch pan (baffles removed). 8 Kg of theophylline 25 mg tablets were subcoated with Advantia Prime 144900BA01 reconstituted at 12% solids under the same standard conditions and spray rate. These tablets were then enteric coated using Advantia Performance 190024HA49, reconstituted at 15%, 20% and 25% solids, and applied to a weight gain of up to 16%. The solution was stirred continuously during the coating run, giving rise to some apparent fluctuation in the spray rate due to the effect of the stirrer and stirring action on the balance. Samples were taken at 2% intervals from 6% weight gain to study the effect of increasing weight gain on the film formation characteristics and the dissolution of the tablets. With the exception of the initial stages of enteric coating process, each run was controlled to achieve the same solid deposition rate. Resultant tablets were stored for at least 24 hours and subsequently subjected to both dissolution testing and TeraView analysis in order to study the potential variable effects on the enteric coat performance.

RESULTS

Enteric Coating Run Data

Dissolution Data

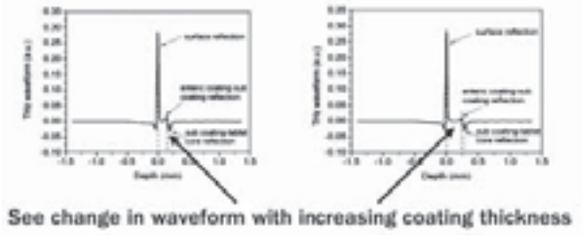
Dissolution Data of Engraved Tablets

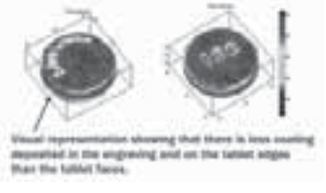


TERAVIEW ANALYSIS

TeraView terahertz technology enables an investigation to be untaken on the structure of oral solid dosage forms. The technique is fast and non-destructive, thus facilitating the analysis of the effects of process changes on the structure of the dosage form and directly correlating these tablet specific changes potentially to other tablet properties such as dissolution or tablet hardness. This paper provides an insight into how terahertz technology can be used to investigate how changes in a coating process subsequently affect film properties. In this case, the primary intent was to increase the coating solids level to reduce the coating process time without negatively affecting enteric performance.

- Typical analysis forms





- Sub coat layer thickness compared to enteric coating layer
- Terahertz map for 16% weight gain at 15, 20 and 25% solids levels
- Effect of solids level and % weight gain on enteric coating thickness

Analysis of Tablet Coating Thickness

Solids level (%)	Mean enteric weight gain (%)	Tablet surface	Mean enteric layer thickness	SD (microns)	Mean sub coat layer thikness (microns)	SD (microns)
15	10	Face	124.43	16.08	33.79	8.70
		Wall	68.35	15.32	33.55	1.51
15	16	Face	194.85	10.32	44.87	7.61
		Wall	117.67	17.08	33.84	1.08
20	10	Face	136.06	15.27	45.00	5.28
		Wall	78.28	10.17	34.20	1.32
20	16	Face	203.28	23.78	50.39	4.32
		Wall	123.20	16.71	37.39	2.19
25	10	Face	127.44	16.57	42.97	4.18
		Wall	67.133	11.76	32.87	1.03
25	16	Face	201.06	26.40	49.22	6.89
		Wall	112.64	16.73	36.11	2.02

All of the tablets coated at 15% solids revealed additional “structure” or “features” within the enteric coating layer. This was not seen when applying the enteric coating at the other solids levels. This effect is seen at both the 10% and 16% coating levels. After examining the process conditions used for 15% solids coating compared to the other solids levels, it is likely that the lower solids coating, having a lower viscosity, produced a higher effective spray rate, potentially leading to increased moisture entrapment within the film or a “moisture induced” physical change in the film structure.

CONCLUSIONS

- Good enteric results were achieved under all process conditions, even with the engraved tablets (which needs further investigation).
- Changing the solids content of the enteric coating suspension did not negatively affect the enteric results, a result that also demands further investigation.
- The TeraView images show some variances in the coating structure as the process conditions change but expanded dissolution studies need to be performed on these tablets to understand if this is significant.
- The TeraView imaging techniques shows a change in the film coat structure when the Advantia Performance was reconstituted at 15% solids, in that it appears that there was entrapped moisture which has allowed some migration of the sub-coat into the enteric coat.
- Further work is required to assess if there are any stability issues associated with the changes in film structure that the TeraView imaging has highlighted.

PO110

Barrier properties of new nanostructured pharmaceutical coatings

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INTRODUCTION

Nanostructured materials are increasingly used by pharmaceutical technology, however no nanocomposites are offered for tablet or pellet coating in spite of the fact that the barrier properties or the drug release controlling function of the coating could be enhanced efficiently by nanoparticles [1-2]. In this work montmorillonite (MMT) nanoparticles have been used for enhancing gastro-resistant and moisture barrier capability of the coating layers around tablets. The active ingredients of pharmaceutical tablets degrade very often under the effect of the humidity of the air [3]. Therefore to ensure the stability of tablets moisture barrier coating should be used. Earlier the moisture barrier coating was manufactured by water insoluble polymers, which require organic solvents and hindered the dissolution rate of the drug [4]. Nowadays water soluble polymers are offered for moisture barrier coating as well, but their barrier capability or their technological properties require improvement.

EXPERIMENTAL METHODS

• Materials

Montmorillonite was supplied by R.T. Vanderbilt Co., Eudragit L30 D55 by Degussa Ltd and polyvinyl alcohol (PVA) based coating material Kollicoat Protect was provided by BASF Ltd.

• Preparation of films

Tablets were coated by Glatt GC 250 pan-coater.
Free films were prepared by the same method as tablet filmcoating. Coating liquids were sprayed onto a Teflon strip placed into the pan of the Glatt coater.

• Gastro-resistant properties of coatings

Coated (Eudragit L30 D55 + different excipients) tablets were added to HCl solution (Erweka DT 6, 37°C, pH=1) and after 2 hours stirring the weight gains of tablets were measured (Radwag WAS).

• Moisture barrier properties of coatings

The moisture absorption of the uncoated and coated tablets were measured (Radwag WAS weighing equipment) after 24 hours precondition in a 33% RH chamber in a chamber of 75% RH at room temperature.

• Disintegration of gastro-resistant coated tablets

The disintegration time of the coated tablets were measured by Erweka DT 6 apparatus according to Ph. Eur. in phosphate buffer (37°C, pH=7.2).

• Distribution of excipients in the films

Free films and coated tablets were investigated by Raman-microscopy (LabRam microscope by Jobin Yvon) and by scanning electron microscopy (SEM) using energy dispersion spectroscopy (EDS) technique (JEOL JSM-6380LA electron microscope). Morphology of the surface was examined by optical-, and atomic-force microscopy (AFM).

• Characterization of coating thickness

Coating thickness was characterized by a non-destructive method (Raman-microscope).

RESULTS AND DISCUSSION

• Moister adsorption

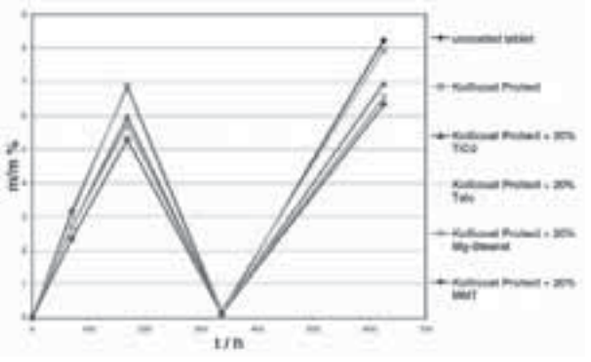

Figure 1: The moisture absorption of the uncoated and coated tablets after 24 hours precondition in a 33 % RH chamber in a chamber of 75 % RH

• Disintegration of gastro-resistant coated tablets

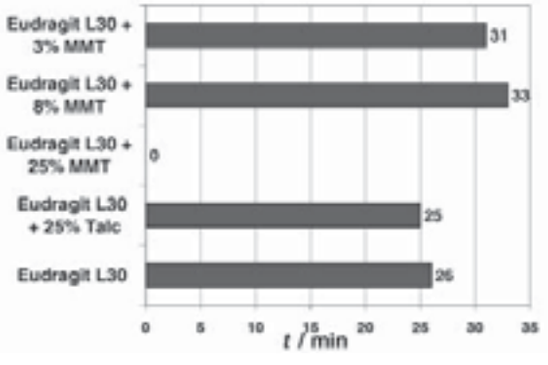

Figure 2: Disintegration time of coated tablets (Phosphate buffer 37 C, pH=7.2) after stirring 2 hours in HCl solution.

Disintegrations of the tablets are extended by montmorillonite.

• Distribution of montmorillonite

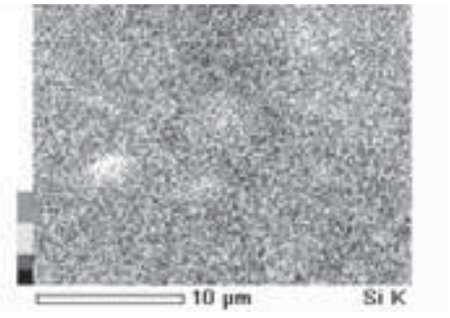

Figure 3: Surface distribution of montmorillonite by SEM EDS.

Distribution of montmorillonite platelets on the surface of the coating was investigated by scanning electron microscope (by using energy dispersion spectroscopy technique) and Raman-microscope.

• Characterization of coating thickness

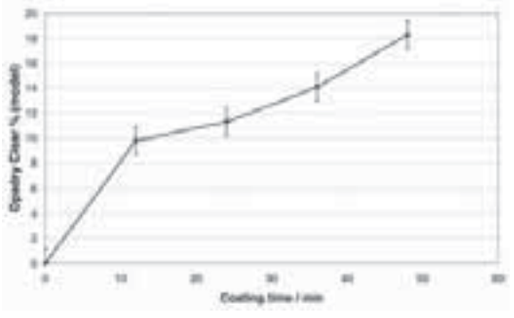

Figure 4: Correlation between Raman spectra and coating time.

The coating quantity of the coated tablet could be characterized by Raman-microscope and Labspec 4.02 on the base of the spectra of the core and the film.

CONCLUSIONS

Different excipients were applied in the well established polyvinyl alcohol based pharmaceutical coating (Kollicoat Protect) and all of them enhanced the moisture barrier properties of the film coatings. However, using montmorillonite in gastro-resistant coating does not meet the requirements. The surface characteristics of the coatings, were characterized by SEM EDS, Raman, optical and atomic-force microscopy, confirmed the uniformity of the coating layer. For the measurement of the coating thickness a non-destructive method was developed by Raman microscope as well, that seems to be appropriate for on-line control of the coating process (PAT).

ACKNOWLEDGEMENT

The work was financially supported by Public Benefit Association of Sciences and Sport of the Budapest University of Technology and Economics and Hungarian State GVOP/3.1.1.-2004-0531/3.0.

References

1. M. Kokabi, M. Sirousazar, Z.M. Hassan, Eur. Polymer. J. 43, 773-781, 2007.
2. C. Verdun, P. Couvreur, H. Vranckx, V. Lenaerts, M. Roland, Journal of Controlled Release, 3, 205-210, 1986
3. G. Zografi, B.C. Hancock, Top. In Pharm. Sci., 405–419 E, Stuttgart, Medpharm 1994.
4. E.Pandula, G.Takács Industrial pharmaceutics, 683-714 p. Medicina Press. Budapest, 1964.

PO111

Pharmaburst ™ and F - Melt® as new granular excipient systems for ODTs preparation

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INTRODUCTION

In the recent years, there has been a growing interest in the orodispersible preparations. Orodispersible tablets (ODTs) are one of the solid dosage forms which can be taken in case of swallowing difficulties. After placing in the oral cavity the tablets disperse or dissolve rapidly in the saliva. According to the requirements of the European Pharmacopoeia 6^{-th} edition their disintigration time should not be longer than 3 minutes. The tablets can be taken with a small amount of water or without water. Furthermore, among orodispersible tablets either IR or CR formulations can be found. Therefore, ODTs are considered as suitable especially in case of a long-term treatement and they are recommended for children as well as for elderly patients [1].

A lot of methods used for ODTs manufacturing have already been described. The most important are freeze-drying, moulding and compression. In spite of that a direct compression consists of the most preferable method becouse of its simplicity [1].

Thus, the aim of the present study was to evaluate the new co-spray dried excipient systems to form ODTs by the direct compression.

MATERIALS AND METHODS

New co-spray dried excipient systems Pharmaburst™ (SPI Pharma, USA), F-Melt® type C, F-Melt® type M (Fuji Chemical Industry Co., Ltd., Japan) were chosen to form a fast-disintegrating tablet matrix. The excipients used in the study are co-spray dried formulations composed of carbohydrates, disintegrants and inorganic ingredients. 2% of Pruv® (stearate fumarate sodium) JRS Pharma, Germany, was used as a lubricant. The excipients were blended with the lubricant for 5 minutes. Tablets of 400 mg were compacted on a single-punch tablet press Korsch by using 12 mm flat-faced punches. The compression force ranged from 5 to 30 kN.

To evaluate the excipients the powder flow properties, the particle size distribution, the specific surface area, H^1-NMR and FT-IR spectra were analysed. The shape of the particles was evaluated by using SEM microscopy. Taking into account disintegration time, hardness, porosity and pore size distribution the quality of the tablets obtained were investigated. Furthermore, the compression behaviour of the materials was also examined.

The mean disintegration time (n=6) was determined in minutes using the disintegration test apparatus (Erweka ZT3, Germany).

A hardness tester (Vanderkamp Benchsaver, Germany) was used to measure the crushing strength of tablets (n=5). Specific crushing strength (SCS) was calculated on the base of the crushing strength, the diameter and the height of the tablet [2].

Porosity (P) of the tablets was calculated from absolute density (ρ) and apparent density (ρ_p). The apparent density was calculated on the base of diameter, height and mass of the tablet. Dimesions of the tablets were measured immediately after the ejection.

Pore size distribution of the best tablets was investigated by mercury porosimetry using PoreMaster 60 (Quantachrome, USA) device. The measurement range of the pore size was from 3,5 nm to 300 μm. The absolute density of samples was determined with helenium pycnometer AccuPyc 1330 (Micromeritics, USA). The specific surface area of investigated samples was determined using the volumetric adsorption analyser ASAP 2010 (Micromeritics, USA).

RESULTS AND DISCUSSION

• Particles characterisation

Both FT-IR and H^1-NMR spectra confirmed similarity of the materials. Using SEM analysis it was found that F-Melt® type M had the most spherical particles. The particle size of the powders was about 100 μm. However, the specific surface area of the materials was different: 0,8698 m^2/g F-Melt® C; 1,3842 m^2/g Pharmaburst™; 3,2647 m^2/g F-Melt® M. Thus, it was hypothesized that it could have an impact on either the compression behaviour or the disintegration time of the tablets obtained.

• Compression behaviour

Among tested materials F-Melt® type C had the best ability to form coherent compacts. The compactibility of Pharmaburst™ and F-Melt® type M was almost similar. The influence of the compression force on the apparent density of tablets is shown in Fig. 1.

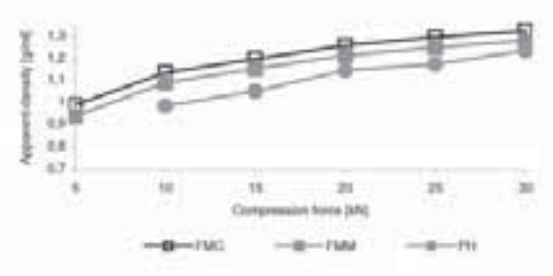

Figure 1: Apparent density of tablets with Pharmaburst™ (PH), F-Melt® C (FMC) and F-Melt® M (FMM).

The porosity of the tablets was about 20% for all optimal formulations. As regards the pore size distribution two main populations of pores were revealed, the first ranged from 0,5 to 5 m, the second consisted of pores lower than 0,01 μm.

• ODTs manufacturing ability

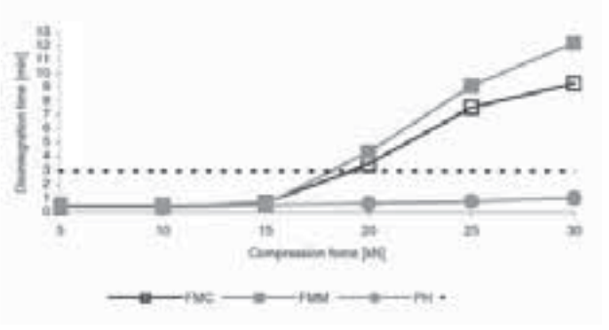

Figure 2: Disintegration time for tablets with Pharmaburst™ (PH), F-Melt® C (FMC) and F-Mel® M (FMM).

It was shown that ODTs can be formed by using either Pharmaburst™ or F-Melt®. All the tablets made of Pharmaburst™ had disintegration time below 3 minutes regardless the compression force (Fig. 2). Moreover, to prepare ODTs of adequate mechanical resistance, it was necessary to apply the higher compression force than in case of F-Melt®. Using Pharmaburst it was impossible to obtain coherent compacts at the lowest compression force. The optimal tablets with F-Melt® can be formed when the compression force ranged from 10 to 15 kN. In case of Pharmaburst™ the compression force from 15 to 20 kN corresponded to optimal process conditions (Fig. 2).

CONCLUSIONS

1. Both Pharmaburst™ and F-Melt® are suitable for ODTs preparation by direct compression.
2. In spite of similar compression behaviour of the materials the disintegration time of the tablets differs.
3. By using Pharmaburst™ it was possible to obtain tablets disintegrating within 3 minutes regardless the compression force.
4. To prepere ODTs of adequate mechanical resistance with F-Melt® it was necessary to apply lower compression force than in case of Pharmaburst™.

5. The shortest disintegration time was noted in case of tablets with Pharmaburst™.
6. Tablets with F-Melt type® C disintegrated more rapidly than those with F-Melt® type M.

ACKNOWLEDGENTS

The authors wish to acknowledge the Fuji Chemical Industry, Japan; SPI Pharma, USA and JRS Pharma, Germany for providing free gift samples of F-Melt®, Pharmaburst™ and Pruv®.

References

1. Y. Fu, Y. Shicheng, S.H. Jeong, S. Kimura, K. Park Orally Fast Disinegrating Tablets: Developments, Technologies, Taste-Measking and Clinical Studies Crit. Rev. Ther. Drug Carrier Syst. 21 433-475 (2004)
2. J.M. Sonnergaard Quantification of the compatibility of pharmaceutical powders Eur. J. Pharm. Biopharm. 63 270-277 (2006)

PO112

Formulation optimization for compressing of coated pellets into orally disintegrating tablets by experimental design and mixture modeling

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INTRODUCTION

There are several clinical advantages of multi-particulate drug delivery systems (MP DDS). They disperse along the GI tract, reduce variations in gastric emptying rates (reduced inter- and intra-subject variability), allow low concentration of bioactive agents locally, and they are less susceptible to dose dumping. In addition, life cycle management of the product is prolonged.

One of the most popular MP DDS developed extensively in the recent years is the orally disintegrating tablet (ODT), a patient-friendly and convenient formulation which can be taken with or without water. Drugs with undesirable properties, such as an irritating or bitter taste, are more challenging to produce in the ODT form, and may require complex technological procedures.

We have developed a technological platform (ODT) containing the drug with the above mentioned properties. The extrusion-spheronization technique was used to prepare drug pellets. The pellets were coated with a taste-masking coating film, and finally compressed into tablets by adding appropriate excipients.

From literature it is well known that compression of coated pellets is a demanding technology [1]; it is of vital importance to select suitable excipients and proper compression parameters.

The present study was designed to obtain the optimal composition of the tablet containing film coated pellets regarding compressibility, hardness, friability, and disintegration time. A constrained full-factorial experimental design study was conducted. A response surface was fitted to measurements in form of Kronecker mixture polynomials [2], allowing the determination of optimum proportions of excipients.

EXPERIMENTAL METHODS

• Preparation of coated pellets

Drug pellets were prepared by extrusion and spheronisation (An axial screen EXTRUDER: Pharmex 35T, Wysstec, Pery, Switzerland, equipped with a 0.4 mm thick screen with 0.35 mm diameter circular openings. SPHEROMAT 250T, Wysstec, Pery, Switzerland.) Pellets were coated in a fluidized bed apparatus (bottom spray i.e. Glatt GPCG 1, Wurster insert).

• Experimental design and optimization of tablet composition

The experimentally varied excipients were chosen to be Mannitol, Pearlitol SD 200, and Pearlitol DC 400, which are essentially mannitol powders with different particulate properties. By choosing appropriate relative amounts, and thereby creating an optimum effective particle size distribution, we have intended to simultaneously achieve fast disintegration, high tablet hardness, and low friability.

Furthermore, the goal was to reduce the risk of segregation by combining excipients of similar sizes as that of the pellets, and by controlling the resulting powder flowability.

Granulates of samples were prepared according to the composition presented in table 1. The weight fraction of coated pellets in the tablet was fixed at 32.86%, and that of additional excipients at 12.4%. The experimental setup follows a constrained 3-factor, 3-level full-factorial design, with the total amount of varied excepient fractions kept constant.

Table 1: Excipient composition of different samples prepared according to the experimental-design study.

Sample No.	Mannitol (%)	Pearlitol SD200 (%)	Pearlitol DC 400 (%)
1	0	0	54.74
2	0	27.37	27.37
3	0	54.74	0
4	8.5	0	46.24
5	8.5	23.12	23.12
6	8.5	46.24	0
7	17	0	37.74
8	17	18.87	18.87
9	17	37.74	0

Excipients and coated pellets were homogeneously mixed and compressed into tablets of mass of 500 mg. The bulk and tapped density of the blend were determined and the compressibility index was calculated. In addition, hardness, friability, and disintegration of the obtained tablets were measured.

The measurements were used to fit a response surface for each of the parameters, which was modeled by 2nd degree homogeneous Kronecker mixture polynomials. The model coefficients were obtained by minimization of prediction error.

RESULTS AND DISCUSSION

The results of measurements of tablet samples are presented in table 2. The fitted response surfaces for hardness, disintegration, friability and compressibility are shown in figure 1. The obtained R^2 values for the models are 0.998, 0.915, 0.888, and 0.573 for hardness, friability, disintegration, and compressibility, respectively.

Table 2: Results of physical evaluation of tablets properties.

Sample No.	Hardness (N)	Disintegration (s)	Friability (%)	Compressibility (%)
1	40	15.5	2.89	14.45
2	62	28.0	0.61	12.75
3	83	28.0	0.70	15.95
4	43	26.0	1.90	13.46
5	60	25.0	1.07	12.76
6	77	30.0	1.16	11.97
7	40	19.5	2.06	16.28
8	52	17.0	0.92	10.31
9	65	17.0	0.66	14.44

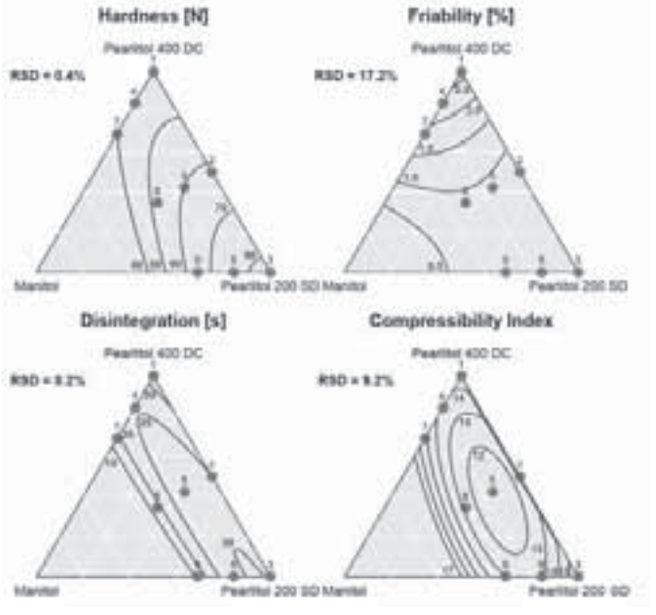

Figure 1: Fitted response surfaces represented by lines of constant hardness, friability, disintegration time, and compressibility.

Model fit quality for tablet parameters of hardness, friability, and disintegration is satisfactory. Using 6 degrees of freedom over 9 data points resulted in acceptable prediction errors, the errors being comparable with the estimated measurement uncertainty. A slightly inferior model quality for compressibility could be explained by the difficulty of accurate measurement of bulk and tapped densities.

A high amount of Pearlitol 200 SD is beneficial both for hardness and friability. The maximum tablet hardness was achieved with only Pearlitol 400 DC, and minimum friability would be achieved using only Mannitol.

The expected correlation of hardness and disintegration is reflected by the similarity of the response contours. Increasing the amount of Mannitol or Pearlitol 400 DC results in faster disintegration times. In both cases the effect of the amount of Mannitol is larger.

Interestingly, for compressibility there is a minimum attainale value at equal proportions of Pearlitol 200 SD and Pearlitol 400 DC, and 8.5% of Mannitol. This could be explained by higher particle packing efficiency. Within the sampled region, compressibility is highest when only Pearlitol 200 SD is used.

From these results the following final mixture of excipients was chosen: 8.5% of Mannitol, 46.24% of Pearlitol 200 SD, and 0.0% of Pearlitol 400 DC. This resulted in a good compromise between hardness and disintegration, produced acceptable friability values, and maintained a satisfactory powder flowability.

Additionally, from the dissolution test of API from final tablets it was also concluded, that the pellet film coating was not noticeably damaged during the compression phase, indicating that the chosen excipients are compatible with pellets.

CONCLUSION

The results of this study indicate that compression of coated pellets into ODT is feasible. By systematically studying the effect of formulation on tablet properties by an experimental design study it is possible to select an optimum composition.

In case of conflicting product goals (such as high hardness and quick disintegration simultaneously), a compromise can be achieved, with sufficient freedom left to optimize the remaining goals.

References

1. El-Mahdi M. and Deasy P.B. Tableting of coated ketoprofen pellets. J. Microencapsulation, Vol. 17, No. 2: 133-144 (2000).
2. Draper N.R. and Pukelsheim F. Mixture models based on homogeneous polynomials. J. Stat. Plan. Infer.,Vol. 71, 303-311 (1998).

PO113

Orally Disintegrating Tablet (ODT) - A novel alprazolam formulation

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INTRODUCTION

In recent years there has been an expansion in development of orally disintegrating tablets (ODT) in the pharmaceutical industry. This mode of administration was initially expected to be beneficial to pediatric and geriatric patients, to people with conditions related to impaired swallowing, and for treatment of patients when compliance may be difficult (e.g., for psychiatric disorders).

Recent market studies indicate that more than half of the patient population prefers ODTs to other dosage forms and most consumers would:

- ask their doctors for ODTs (70%),
- purchase ODTs (70%)
- prefer ODTs to regular tablets or liquids (>80%)

DEFINITIONS

General: An ODT is a solid dosage form that disintegrates and dissolves in the mouth without water within 60 seconds or less.
The US Food and Drug Administration Center for Drug Evaluation and Research (CDER) defines it in the Draft Guidance for ODT as "A solid dosage form containing medicinal substances, which disintegrates rapidly, usually within a matter of seconds, when placed upon the tongue". Non-binding recommendations are also: disintegration time less than 30 sec. and a tablet weight up to 500 mg.

The European Pharmacopoeia however defines a similar term, orodispersible tablet, as an uncoated tablet that can be placed in the mouth where it disperses rapidly before swallowing.

ADVANTAGES

Clinical; Improved drug absorption, faster onset of action, minimized first-pass effect, improved bioavailability.

Medical; No tablet or capsule swallowing, improved taste, no water needed, improved safety and efficacy, improved patient compliance.

Technical; Manufactured with common process and conventional equipment, can use sugars and other excipients generally recognized as safe, more accurate dosing compared to liquid products.

Business; Extended patent protection, marketing exclusivity, product line extension, unique product differentiation.

LIMITATION FACTORS

Clinical; Varying degrees of pregastric absorption depending on the active substance and/or the formulation, varying pharmacokinetic profile, varying bioequivalence compared to the conventional oral dosage form, avoidance of first-pass metabolism may have implications for drug safety and efficacy, inherent patient-to-patient disintegration time variability.

Technical; Difficulty of incorporation of high doses of active substance, achieving fast disintegration, successful taste masking and achieving satisfying organoleptic characteristics, having a standardized disintegration/dissolution method.

ODT MANUFACTURING TECHNOLOGIES

Wowtab® Direct compression of new physically modified polysaccharides that have good water dissolution characteristics that facilitate fast disintegration and high compressibility. The manufacturing process involves granulating low-moldable sugars (e.g., mannitol, lactose, glucose, sucrose, and erythritol) that show quick dissolution characteristics with high-moldable sugars (e.g., maltose, sorbitol, trehalose, and maltitol).

Orasolv® Direct compression of active ingredients, effervescent excipients, and taste-masking agents. The tablet quickly disintegrates because effervescent carbon dioxide is produced upon contact with moisture.

Durasolv® Direct compression of noncompressible fillers with a taste-masking excipient and active ingredient into a dry blend. The blend is compressed into tablets using a conventional rotary tablet press. Tablets made with this process have higher mechanical strength and are sufficiently robust to be packaged in blister packs or bottles.

Lyophilization (Zydis® and Lyoc®) Freeze-drying process that involves removal of water (by sublimation upon freeze drying) from the liquid mixture of drug, matrix former, and other excipients filled into preformed blister pockets. The formed matrix structure is very porous in nature and rapidly dissolves or disintegrates upon contact with saliva. The Zydis technology requires specific characteristics for drug candidates and matrix-forming materials. Drug loading for water-insoluble drugs approaches 400 mg, and the upper limit for water-soluble drugs is ~60 mg. Ideal drug candidates for this method are insoluble drugs that have low water solubility, have fine particle size, and aqueous stability in the suspension.

MATERIALS:

- ODT Formulations- A and B: Fast dispersible excipient based on mannitol, polyvinyl acetate and crospovidone; (Ludiflash® BASF AG), crospovidone (Kollidon CL-SF ®, BASF), Sodium stearyl fumarate (Pruv®, JRS).
- Immediate Release -Formulation Ref: microcrystalline cellulose PH102 (Vivapur102® JRS, Germany), Starch Pregelatinized

(Starch 1500®, Colorcon LTD), Lactose Monohydrate (Tablettose 80®, Meggle GmbH), crospovidone (Kollidon CL-SF ®, BASF), docusate sodium (DSS, Laxachem Organics LTD), magnesium stearate (Mosselman).

Tablet composition (%)

	Formulation A	Formulation B	Formulation Ref.
Alprazolam	0.77	0.77	0.77
Ludiflash®	97.73	96.73	Other ingradients
Kollidon CL-SF	/	1	1.5
Pruv®	1.5	1.5	/
Mg-Stearate	/	/	1.2
Tablet weight	130 mg	130 mg	130 mg
Tablet form	7 mm, round, biconvex	7 mm, round, biconvex	7 mm, round, biconvex
Tablet Hardness (kp)	6.2	6.4	6.5
Carr s index (%)	16	16	21
Disintegration time	10 sec	8 sec	5 min
Dissolution	94.52 %	92.85 %	100.22 %

MANUFACTURE OF TABLETS

Direct Compression:

All excipients except lubricant were manually sieved through 0.8 mm sieve, and mixed in a polyethylene bag for 5 min. Lubricant was added, mixed for 2 min. and tablets were produced by direct compression on Erweka AR 402 single punch tablet press, with constant compression force. Physical parameters of the dry mix and tablet properties were recorded and evaluated.

TEST METHODS

Disintegration testing: As specified in USP30-NF25, test method 701. Dissolution testing: As specified in USP30-NF25, dissolution method for Alprazolam tablets; Buffer solution pH=6, 500 ml, Apparatus 1, 100 rpm, 30 min. Tolerances; Not less than 80% (Q) of the labeled amount of Alprazolam is dissolved in 30 min.

RESULTS AND DISCUSSION

- It is a challenge to formulate a low soluble API (active pharmaceutical ingredient) into an ODT dosage form, due to the low amount of water present in the oral cavity, which could limit the disintegration time and oral bioavailability of the API.
- It is shown in the results above that an ODT dosage form can be successfully formulated with Ludiflash®, with very short disintegration time and dissolution requirements met according to Pharmacopoeial tolerances.
- % Dissolution of the reference formulation is higher than the ODT formulations A and B. This is due to the presence of solubility increasing agent in the reference formulation and absence of solubility enhancer in both ODT formulations. This could present a difference for the 2 dosage forms in a bioequivalence study. On the other hand due to an ODT formulation, there is the possibility of improved drug absorption (upper parts of GIT), minimized first-pass effect and consequently improved bioavailability.
- Alprazolam is an anxiolitic and anti-depressant agent, and due to it's therapeutic categorization, it is highly advantageous for patients to have this agent formulated as an ODT.

References

1. US Food and Drug Administration, Gudance for Industry- Orally Disintegrating Tablets, April 2007
2. European Directorate for the Quality of Medicines, , European Pharmacopeia 5.2, 3151 (2006).
3. K. Cremer, "Orally Disintegrating Dosage Forms Provide Life Cycle Management Opportunities," Pharm. Technol. Formulation and Solid Dosage 2003, 22–28 (2003).

PO114

Formulation of tablets with probiotics

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INTRODUCTION

The term »probiotic« represents organisms and substances which contribute to intestinal microbial balance, defined by Parker in 1974. Although resident flora, that has an important protective function, is relatively stable, it may be influenced by several dietary and environmental factors. Preparations containing viable probiotics, as lactic acid bacteria (LAB) appear to have value in restoring normal microbial function and alleviating symptoms in some patients suffering from gastrointestinal infections and other disease conditions. A prerequisite for any effect of ingested bacteria is a successful transit and implantation in the gastrointestinal tract. Based on the acid instability of LAB it is essential to consume these probiotics with food or to deliver them by appropriate pharmaceutical formulation [1].

The aim of this study was to prepare lyophilized product of LAB that can be included in tablet preparation. It was considered a guide of the International Dairy Federation that recommended the presence of app. 10^8 viable bacteria in the product to assure appropriate activity. Additional goal in formulation and evaluation processes was to allow a decrease of viable cells of not more than one log-unit. Dry product of lactic

acid bacteria (LAB) was used to formulate gastric juice resistant tablets where matrix forming component polyacrylic acid (PAA) was combined with sodium alginate (SA) as well. To optimize the formulation - using survival rate in acid medium and disintegration time in intestinal fluid as test parameters - tablets with constant LAB content were modified with respect to amount of excipients used per tablet and compaction forces.

EXPERIMENTAL METHODS

• ***Bacteria growth conditions.*** The probiotics, lactic acid bacteria (LAB) (Lactobacillus acidophilus), were cultured in MRS broth (Merck, Darmstadt, Germany) for 24 h at 37°C.

• ***Lyophilization.*** Cell suspension was freeze dried for 24 h at 0.12 mbar (Beta 1-8K, Christ, Germany) and survival of LAB after lyophilisation was determined.

• ***Preparation of tablets.*** All tablets weighted 300 mg and contained constant quantity of LAB (150 mg) and lubricants (3 mg) as well. Amount of excipients, PAA and SA, varied, as shown in Table 1. Weighed ingredients were individually compressed into tablet using a single punch tablet press (Korsch EKO, Berlin, Germany) with 12 mm diameter flat punches. In one formulation compaction force (kPa) was modifying during the tableting process in spite to investigate its influence on survival of LAB (Table 1).

Table 1: Overview of all examined tablets.

Formulation	LAB : PAA : SA (w/w) (%)	Compaction force (kPa)
A	50 : 25: 25	0.5 - 2.0 - 8.5
B	50 : 12.5 : 37.5	0.5
C	50 : 50 : 0	0.5

• ***Resistance of tablets against acid conditions.*** Tablets were exposed to the acid pH, similar to the gastric one (0.04 M HCl; pH 1.9) (G-pH), for 2 h at 37°C and after that remnant of tablet (in all cases only slightly swollen tablets) were dissolved in PBS (pH 7.4), to release incorporated LAB, and determine survival of bacteria.

• ***Disintegration of tablets in the fluid of intestinal pH.*** After 2 h of acidic conditions tablets were removed into fluid of intestinal pH (phosphate buffer; pH 6.8) (I-pH), left until complete disintegration and finally determine the survival of released LAB.

• ***Survival of LAB.*** Influence of lyophilisation process, compression and media of different pH on survival of LAB was monitored using a bioluminescence ATP assay (Sigma, Chemical Co., ZDA). LAB suspension, gained with dispersion of lyophilized product or complete disintegration of tablets, was used to quantify intracellular ATP, which was released after LAB lyses. Number of viable LAB was calculated regarding previous determined amount of ATP per cell. Results are represented as log-value of received LAB-number.

RESULTS

Freeze drying is a process that may cause injury and death of cells [2]. Subsequently number of LAB after lyophilization process decreases for a twice.

Lyophilized LAB when compressed at the constant compaction force (0.5 kPa) with different ratios of excipients showed similar loss of viable bacteria, what indicates insignificant effect of chosen excipients ratios on survival rate (Fig.1).

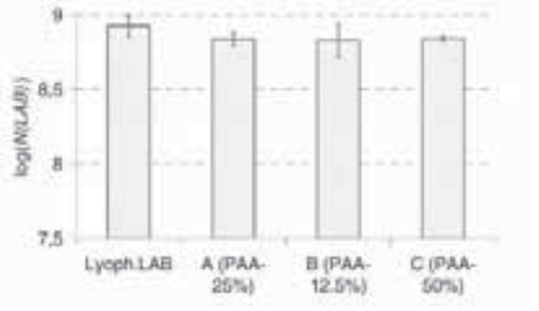

Figure 1: Survival of LAB after constant compaction force (0.5 kPa) and different excipient ratios (see Table 1).

However, importance of excipient ratios is exposed in dissolution assays. Formulation with the highest amount of PAA ("C") required almost 8 h for disintegration in the intestinal pH (pH 6.8). What is more, high amount of PAA (tablet "C") weaken resistance against acid pH (G-pH) (Fig. 2). Well-known swelling–deswelling process of PAA under acidic pH [3] resulted in formation of huge pores in tablet matrix that enable fast release of LAB and final low survival (Fig. 2). Following dissolution of tablet "C" at intestinal pH (pH 6.8) resulted in rapid erosion. Consequently higher survival was received at pH 6.8 (I-pH) than at pH 7.4 (PBS, after pH 1.9 – G-pH) (Fig. 2).

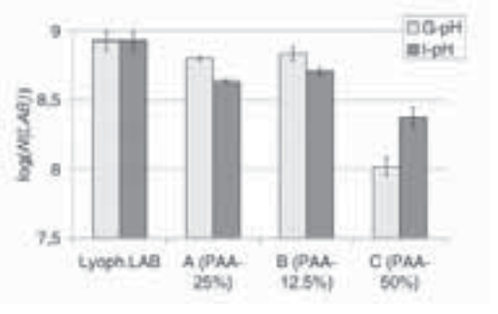

Figure 2: Resistance against acid conditions (G-pH; bright columns) and final survival after tablet disintegration in a fluid of intestinal pH (I-pH; dark columns) regarding the excipient ratios (see Table 1).

Adding SA to PAA strengthens tablet matrix. At acidic pH SA is converted to insoluble alginic acid [4]. Therefore, only slightly swelling was observed for tablets "A" and "B" in acid media, resulting in slow release of LAB and consequently higher survival compared to tablet "C" (Fig. 2). Followed dissolution of tablets "A" and "B" in a fluid with intestinal pH (I-pH) showed slightly decrease of bacteria survival (Fig. 2). Disintegration time of these tablets was around 3 h, what indicate acceleration in disintegration when compared to tablet "C".

Compression of formulation "A" at higher compaction forces (1.8 and 7.8 kPa) led to decreased survival of bacteria as was expected (Fig. 3). Disintegration time increases only trifle with strengthening the com-

paction force. However, the highest compaction force (7.8 kPa) resulted in insignificance oscillating of survival what suggests formation of the strongest matrix structure that successfully resist to the invasion of surrounding media.

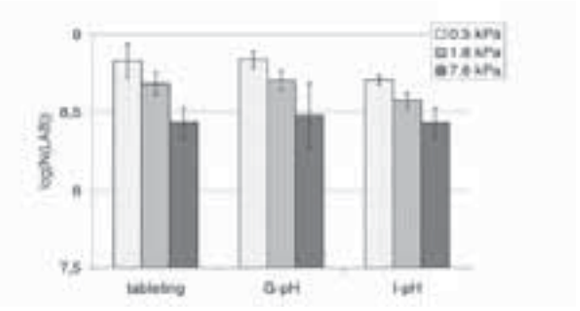

Figure 3: Influences of compaction forces on survival rate of LAB in tablet "A" after tableting and exposure to G-pH or I-pH.

CONCLUSION

In the present study, the tablets resistant against acid pH were prepared that enable the delivery of 10^8 -10^9 viable bacteria to the intestine. Excipients used were not decisive for survival of LAB in tableting process. However, the ratio of PAA to SA was important to achieve acid resistance and disintegration time around 3 h at intestinal pH. The compaction force was also shown to be a key parameter affecting survival of bacteria and should therefore be monitored.

References

1. M. Stadler et al, Int. J. Pharm. **256** 117-122 (2003)
2. G. Zarate et al, Process Biochem. **41** 1779-1785 (2006)
3. Y. Yin et al, Carbohydr. Polym. **71** 682-689 (2008)
4. A.L. Ching et al, Int. J. Pharm. **355** 259-268 (2008)

PO115

The investigation of the sugar hot melts

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INTRODUCTION

Sugar hot melts are gaining increasing recognition as effective delivery systems in the production of solid pharmaceutical forms. In the form of lozenges or medicated lollipops, they enable effective, safe, sustained and continued delivery of numerous drugs, such as vitamins, minerals, immunomodulating agents, analgetics, anaesthetics, antimicrobial agents, antiseptics, antitusics, adstringents, corticosteroids and hormones, to achieve the local healing in the mouth or throat and to succeed the systemic effects (1,2).

Although the majority of products are subjected to numerous pharmacological and stability testings during the process of the development of new formulations, the manufacture and development of hard candy bases (sugar hot melts) still largely depend on experience and intuition, as the structure and the influence of composition and production parameters on the quality of products made from hard candy bases have not been sufficiently researched as of yet. An essential condition for making quality products with an appropriate shelf life from hard candy bases is the manufacture of a melt that contains a minimum level of residual water coupled with balanced levels of saccharosis and sugars that inhibit crystallization (for example starch syrup). The manufacture process itself is essentially based on the transformation of a mixture of saccharosis and starch syrup, which is heated up in vacuum, into a hard candy base with low water content. Following the addition of drugs and other additives, an amorphous glassily structure forms during the process of cooling (3).

Amorphous structure substances exhibit a characteristic glass transition temperature (Tg) that can be affected through the addition of plasticizer and antiplasticizer. As the Tg of the hard candy base approaches the temperature conditions of production, certain problems begin to manifest (agglomeration, crystallization). The purpose of the research was to specify the physical-chemical structure of the hard candy base throughout the entire process of manufacture, and establish the effect of the degree of vacuum maintained during the process, and the water content in the melt, on its Tg. Furthermore, we examined the effect of selected additives on the thermal behaviour of cooled melts.

EXPERIMENTAL METHODS

We examined samples of hard candy bases without additives (composed only of sugar, starch syrup, lactose and sorbitol) at various degrees of vacuum, and samples with added selected substances in concentrations that correspond to those usually found in products: vitamin C, vitamin B_2, vitamin B_6, nicotinamide, vitamin A, vitamin E, citric acid, polyvinyl pyrrolidone K25. The water content of the samples was established using the Karl Fischer and NIR methods. During the examination of the structure of samples we used several techniques for assessing thermal transitions (DSC, MTDSC), while the spectroscopic technique employed was FTIR. Additionally, samples have been examined with optical microscopy. By way of examining the dynamic vapour sorption, we attempted to establish and assess the preliminary sorption isotherms of certain samples.

RESULTS AND DISCUSSION

It has been found that the effectiveness of the vacuuming process in the beginning stages of production shows a characteristic effect on the water content of hard candy base and subsequently on its Tg (Fig. 1). It was established that melts - under given experimental conditions - maintain their amorphous state during all manufacturing stages, regardless of their initial water content level. By tracking the content of water throughout the manufacturing process, it was confirmed that melts with lower initial water content in the hard candy base exhibit a larger affinity for binding water in the subsequent process of manufacture. In spite of the above, the initial content of water and the degree of vacuum used during the preparation of the melt still have the largest and essential effect on the water content in the product.

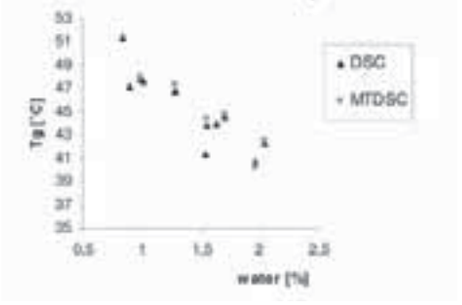

Figure 1: The effect of water content on Tg

It has been observed that the addition of vitamins generally decreases Tg values of cooled melts. Hydrophilic vitamins have a more significant effect. It is assumed that they are incorporated more easily into the sugar matrix due to their structural similarity, which is an essential condition for plasticizer. The effect depends additionally on the amount of the additive used. Among the tested hydrophilic vitamins, vitamin C exhibited the greatest effect at the given concentration. The Tg was also characteristically decreased upon the addition of citric acid (Fig. 2).

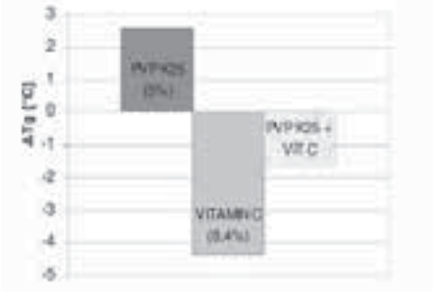

Figure 2: Influence of vitamins (and citric acid) on Tg

It has been confirmed that the effect of additives will be manifesting to a greater degree when the initial water content is lower.

It was further established that the addition of the PVP decreases the Tg of the examined hard candy base, in a manner similar to that already established in connection with PVP colyophilisates with certain sugars (4) – acting as an antiplasticizer. If the combination of Vitamin C and PVP (plasticizer and antiplastizicer) was used, the mutual compensation effect was confirmed (Fig 3).

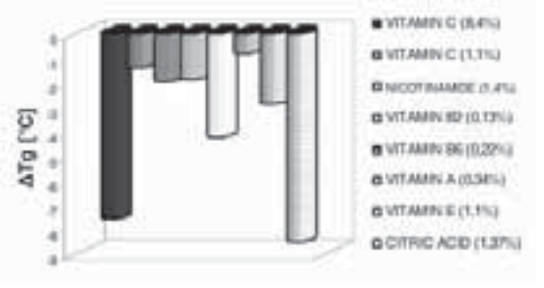

Figure 3: PVP decreases the effect of the plasticizer

CONCLUSION

It was showed that the structure of hard candy base is amorphous throughout the entire production process. The characteristic property of the amorphous structure – Tg – is affected by the content of water and other additives. Water and vitamins, depending on their concentration levels, decrease the Tg. This effect can be, however, reduced by using antiplasticizer, e.g. PVP K25.

References

1. Allen L V. Compounding hard, soft and chewable Troches/Lozenges/Drops. Int J Pharm Comp, 1999; 3,6:461-465.
2. Hermeilstein N H. Confectionery Processing. Food Technology, 1999; 53,12: 63-65.
3. Edwards W P. The science of sugar confectionery. The Royal Society of Chemistry, Cambridge 2000; 1-88.
4. Zhang J, Zografi G. Water Vapor Absorption Into Amorphous Sucrose – Poly(Vinyl Pyrrolidone) and Trehalose – Poly(Vinyl Pyrrolidone) Mixtures. J Pharm Sci 2001; 90(9): 1375-1385.

PO116

Development of an oral liquid controlled release formulation based on ibuprofen pellets

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INTRODUCTION

Ibuprofen is the active ingredient in a number of pain and antipyretic relievers. It is rapidly absorbed but its short biological half life of 2 h requires a multiple daily dosing. Children, older persons and disabled or incapacitated patients often have trouble swallowing tablets or capsules. So it is desirable to provide the drug in a liquid form. Liquid suspension dosage forms often have stability problems. The drug often settles out as sediment which can be a dosing problem for the patient. Another common problem associated with liquid dosage forms is the disagreeable taste of the drug. The objective of the present study was the development of a ready-to-use liquid controlled release formulation with high stability, low sedimentation and good taste masking characteristics in order to improve the patient compliance. Therefore a thixotropic system was developed in which lipophilic matrices pellets were incorporated.

The rate at which a drug goes into solution is an important determinant for the absorption in the gastro-intestinal tract.

To predict the in-vivo performance of ibuprofen after oral administration it is necessary to understand about the effect of food on the pharmacokinetics of the drug and in particular the composition of the bile salt in the human intestinal fluid. For poor soluble weak acids like ibuprofen, a class II drug, little dissolution occurs in the stomach. The dissolution rate increases with increasing pH so nearly the complete dissolution occurs in the small intestine. Therefore biorelevant in-vitro dissolution tests can be used that are capable of predicting in vivo performance [1,2].

Because of the lipophilic filler used for the pellet preparation and the rheology modifiers used for creating a thixotropic system we investigated the possible influence of the stearate and the Avicel on the dissolution rate of ibuprofen. For the current investigations three suitable media for simulating the composition of the gastrointestinal tract were used.

MATERIALS AND METHODS

Pellets

Different pharmaceutical lipophilic fillers were evaluated for the pellet formulation. The pellets were produced by the extrusion and spheronization technique, dried at 30°C for 24 h and sieved with sieves of 0,1-1,25 mm aperture. Concerning the surface area, particle form, porosity and *in-vitro* kinetic calcium stearate showed the best results.

Thixotropic system

Six different rheology modifiers were incorporated into water respectively into water and xanthan gum in ratios from 1-6 %. A rotational cone/plate rheometer " Physica MCR 501, CP 50-1" was used to evaluate the structure decomposition under high shear load as well as the structure recovery at rest. The recovery times were investigated over a period of 180s. The evaluation showed the percentage regeneration in comparison to the initial viscosity. In series oscillatory measurements were carried out. The frequency sweep characterized the decomposition attitude and long term stability of the formulations. The amplitude sweep was carried out in order to evaluate the linear viscous elasticity area for the frequency sweep. All measurements were performed at 20° C, the shear gap was defined to be 0,048 mm.

In vitro-Release

The pellets were incorporated into the thixotropic systems. To simulate gastro-intestinal conditions the USP XXV rotating basket method was used to determine the dissolution profile of ibuprofen in the USP phosphate standard buffer at pH 6.8. For further investigations three biorelevant media, Simulated Intestinal Fluid (SIF), Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinalfluid (FeSSIF) were used [3,4] .

RESULTS AND DISCUSSION

The data showed that three rheology modifiers from the type Avicel recovered after destruction, the recovery times varied from 60 seconds to 180 seconds. The ratios of about 4 - 6% showed the best results (56 – 82 % structure regeneration). The shear thinning additive xanthan improved the structure regeneration of about 10-15% and decreased the

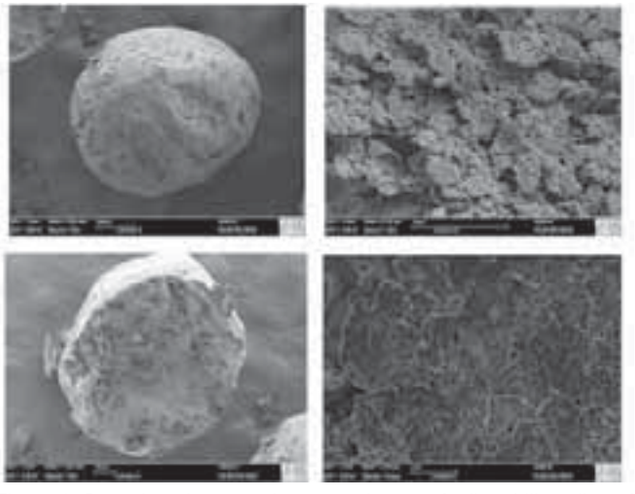

Figure 1: Scanning electron microscopy image of a lipophilic pellet, of a cross cutted lipophilic pellet, of the structure outside, of the structure inside

deformation from 15 % to 7-10 %. This could be explained by a higher elastic amount. Constant structure strength through the whole frequency field and high resting structure strength signify a rigid system. All formulations demonstrated relative constant structure strength. In two of three formulations the resting structure decreased with an increasing xanthan concentration.

The lipophilic pellets were incorporated into three different thixotropic systems, consisting of water/Avicel RC-581, water/Avicel RC-591, water/Avicel CL-611, in amounts of 4 respectively 5 %. Additionally each formulation contained an amount of 0,2 % xanthan. The pellets did not settle out as sediment or buoyed upwards to the surface. After quick shaking of the bottle the viscosity decreased and the suspension was easy to dose.

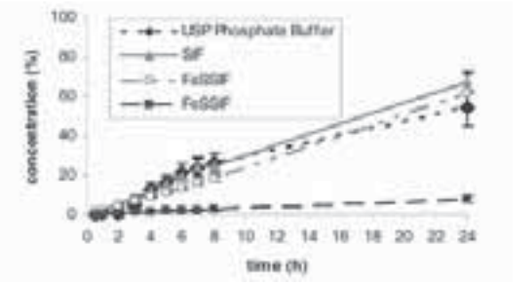

Figure 2: Dissolution profiles if Ibuprofen in various media

The *in-vitro* kinetics in the USP phosphate standard buffer showed that 53,95 % of ibuprofen could be found in the medium after 24 hours. The presence of bile components increased the dissolution rate to an average maximum of 61,1 % and 67,2 % in FaSSIF and SIF, respectively. Changing the medium to FeSSIF, dissolution was slower, with 8,15 % dissolving after 24 hours. The decrease in pH from 7.5 (SIF) and 6.5 (FaSSIF) to 5.5 (FeSSIF) impaired the dissolution drastically.

CONCLUSIONS

The incorporation of lipophilic pellets into a thixotropic system resulted in an oral ready-to-use liquid controlled release formulation. Because of immediate reconstruction of the formulation it is possible to prevent sedimentation of the pellets. The biorelevant dissolution media can be used to predict the in vivo behaviour of the developed formulation.

References

1. Dressman J. B, Reppas C. In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs. Euro. J. of Pharmaceutical Sciences 11: 73 - 80 (2000)
2. Moreno Perez de la Cruz et.al. Characterisation of fasted -state human intestinal fluids collected from duodenum and jejunum. J. of Pharmacy and Pharmacology 58/8: 1079 – 1089 (2006)
3. USP XXVIII 2005 The Official Compendia of Standards USP 28 Edition, NF 23 Edition. United States Pharmacopoeia Convention Inc. Rockville (2005)
4. Marques M. Dissolution Media Simulating Fasted and Fed States. Dissolution Technologies (2004)

PO117

Polysaccharides and polyacrylates in formulation of prolonged-release dosage forms

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INTRODUCTION

The formulation of modified-release dosage forms brings many advantages: the optimization of the pharmacokinetic profile of the drug, the reduction of fluctuations of plasmatic concentration, the increasing of the drug benefit and improvement of the patient compliance.

The matrix system and/or multiple unit dosage form provide the reliable guaranty of controlled delivery of the drug from the modified-release dosage forms. In this study both mentioned formulations were prepared and evaluated. The aim of study was the preparation of the modified-release dosage form of the cardioprotective drug trimetazidine applying the matrix forming properties of the natural polymers and the comparison of natural polymers with the synthetic polymers from the group of polyacrylates.

Two natural polymers were studied: polysaccharides sodium alginate and carrageenan as a hydrophilic matrix forming polymers. They swell in the contact with the body fluids and form the barrier which control the drug release. Alginates are preferably used in controlled-release matrix tablet formulations of drugs with pH dependent solubility. At the low pH in the stomach the alginates form insoluble gel layer that acts as a barrier for diffusion of the drug and thus limiting the release of the drug in the stomach. Upon the passage of the matrix into the higher pH environment of the small intestine the alginates form soluble viscous layer , that still affords a barrier to drug release by diffusion while undergoing erosion and alginates give constant drug release. This minimizes the possibilities of clinical side effects caused by variable release rates due to pH variations in gastrointestinal tract. In case of usage of carrageenans there is necessary to take into consideration their instability in the acidic pH. The coating of the matrix by enterosolvent polymer provides the suitable solution of this problem.

Moreover two polymers from the group of hydrophobic acrylate polymers were studied : Eudragit NE and Eudragit FS which form the mem-

brane that controls the release of the drug. These polymers vary in their solubility, which differently depends oh pH.

EXPERIMENTAL METHODS

The matrix tablets were prepared by direct compression, the following excipients were used: the microcrystalline cellulose Avicel PH 102 as a filler, magnesium stearate as a glidant and colloidal silicon dioxide as an excipient improving flow properties. As the control release polymers sodium alginate Protanal LF 240 D and carrageenan Gelcarin GP 379 NF were used (in amount of 30% to 60 % by weight).

The pellets were prepared by extrusion and spheronisation using the filler Specicel 140, the mixture of microcrystalline cellulose, lactose and sodium salt of carboxymethylcellulose. The pellets were coated by fluid bed technic with suspension of Eudragit FS and Eudragit NE (in amount 4.5% and 9 % by weight) with addition of emollient triethylcitrate.

The controlled release effect of the polymers was evaluated in relation to their functionality, content and pH dependence. The dosage forms were examined in the accelerated stability testing.

The release of the drug was tested by dissolution procedure in buffer solutions (pH 1.2, 4.5, 6.5 and 7.2) with following method parameters: basket apparatus, temperature 37 ± 0.5°C, rotation speed 100 rpm, determination of dissolved amount of drug by UV spectrophotometry at 205 nm.

RESULTS AND DISCUSSION

The evaluated polymers can affect the rate of the drug release from the matrix tablet system and from the reservoir system, too. In comparison with conventional immediate-release formulation of trimetazidine, the release profile of matrix tablets is prolonged to 9 hours (Fig. 1).

Both types of studied polyacrylates (Eudragit NE and Eudragit FS) form the coherent membrane on the microparticles and the release of the drug is modified even at the low content of polymer 4.5% and 9 % by weight (Fig. 2).

The comparison of the drug release from matrix tablet and reservoir system in figure 3 shows, that the dissolution rate from matrix containing 28.6% of polymer carrageenan is comparable with dissolution rate from reservoir system containing only 4.5% of polymer.

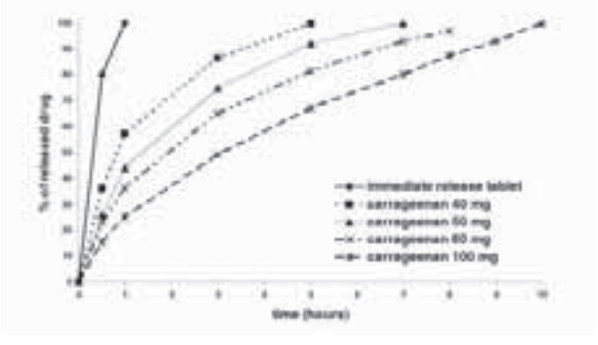

Figure 1: Drug release from the carrageenan containing matrix as a function of polymer content

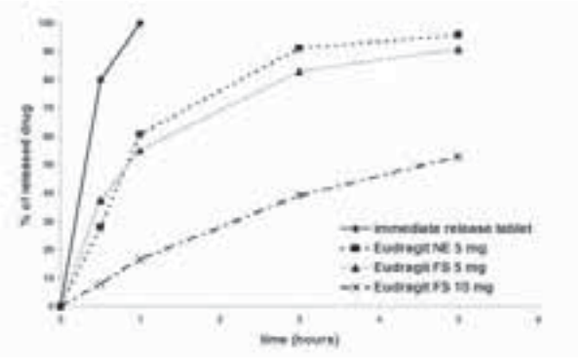

Figure 2: Drug release from polyacrylates coated microparticles

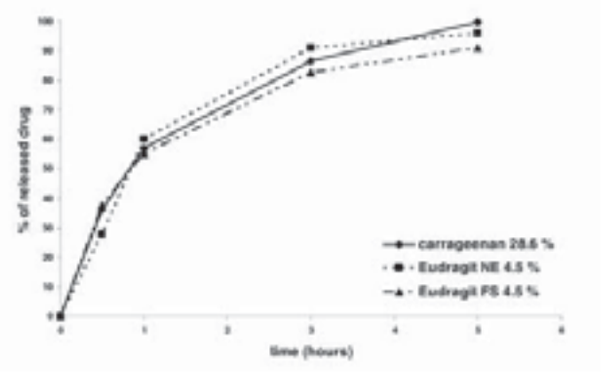

Figure 3: Comparison of drug release from carrageenan matrix tablet and polyacrylates reservoir system

CONCLUSION

The evaluated polymers decreased the release rate of the trimetazidine from the formulation.

They are usable as a control released polymers in the preparation of matrix and reservoir systems. The modification of the release rate was achieved by using of natural polymers and polyacrylates, too. The results showed more sensitive and more effective regulation of the release rate by polyacrylates forming reservoir system , in comparison with matrix system.

References

1. Holte Q., Onsqyen E., Myrvold R., Karlsen J. International Journal of Pharmaceutics 20: 403 - 407 (2003).
2. Tapia C., Ormazabal V., Costa E., Yazdani-Pedram M. Drug Development and Industrial Pharmacy 33: 585 – 593 (2007).
3. Chan L.W., Ching A. L., Liew C.V., Heng P. W.S. Development and Industrial Pharmacy 33: 667 – 676 (2007).
4. Liew C.V., Chan L.V., Ching A.L., Heng P.W.S. International Journal of Pharmaceutics 309: 25 – 37 (2006).
5. Bani-Jaber A., Al-Ghazawi M. Drug Development and Industrial Pharmacy 31: 241 – 247 (2005).
6. Coviello T., Matricardi P., Marianecci C., Alhaique F. Journal of Controlled Release 119: 5 – 24 (2007).
7. Gupta V.K., Hariharan M., Wheatley T.A., Price J.C. European Journal of Pharmaceutics and Biopharmaceutics 51: 241 – 248 (2001).

PO118

Development of sustained release tablet of metformin hydrochloride by aqueous coating

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INTRODUCTION

The oral antihyperglycemic agent metformin hydrochloride (MH) is well established as a therapeutic agent in patients with type-2 diabetes mellitus (T2DM). Oral doses of Metformin are generally recommended in the range of 500 to 2500 mg a day and a single dose may vary from 500 to 850 mg.

RATIONAL AND OBJECTIVE

Metformin is highly soluble in water and can abruptly reduce the sugar level in blood due to rapid release when administered in normal tablets. Therefore not only for convenience of patients but also for efficient treatment, a tablet designed to release the drug content at constant rate. Also it is an objective to reducing the emission of volatile organic compounds by switching from a solvent-based coating to an aqueous-based coating without changing the release profile of a tablet containing highly water-soluble drug Metformin hydrochloride and to minimize hazards associated with using both flammable and potentially toxic solvents.

MATERIAL AND METHODS

Methacrylic acid copolymers (Eudragit® RL30D, Eudragit® RS30D) were supplied by M/s Degussa India Pvt Ltd., Mumbai, India. Talc, Polyvinyl pyrrolidon, polyethylene glycol 400, propylene glycol were obtained as gift from M/s Que Pharma Pvt. Ltd., Wadhwan, Gujarat, India. Metformin hydrochloride was obtained as gift from M/s Cipla Pvt. Ltd., Mumbai, India.

PREPARATION OF METFORMIN HYDROCHLORIDE TABLET

Metformin hydrochloride tablet were prepared by wet granulation method. Metformin hydrochloride, dicalcium phosphate, PVP, magnesium stearate and Talc were used for preparation of tablet. Tablets were compressed by using 12.5 mm standard concave punch. Total weights of the tablet were 650 mg containing 500 mg Metformin hydrochloride. Hardness of tablets was kept at 4-5 kg/cm^2.

CHARACTERIZATION OF TABLETS

The properties of the compressed tablet, such as hardness, friability, weight variation, and content uniformity were determined using reported procedure.

PREPARATION OF COATED TABLETS

Coating dispersion: Coating solution consisted of release retardant polymers Eudragit®-RS30D, Eudragit®-RL30D, Plasticizer Triethyl citrate, Talc. Tablets are coated with different coating levels are as follows;

Tablet coating with RS:RL(5:1)	Tablet coating with RS:RL(3:1)
3 % coat load	3 % coat load
1.5% coat load	1.5% coat load
1 % coat load	1 % coat load

The coated tablets were dried in an oven at 40°C for 12 hr.

DRUG RELEASE STUDIES FROM TABLETS

The release characteristics of uncoated and coated metformin hydrochloride tablet were studied in triplicate using a USP type 2 dissolution apparatus with a stirring speed of 100 rev./min at 37 0.5^{0}C in 900 ml of simulated gastric fluid pH 1.2 for 2 h followed by study in simulated intestinal fluids pH 6.8 thereafter. The aliquots were withdrawn at definite intervals of time. The drug content was determined spectrophotometrically at 232 nm.

RESULT AND DISCUSSION

The tablets coated with Eudragit® RS30D and Eudragit® RL30D in the ratio 5:1 resulting in 1% coat load showed sustained release effect for 12 hr, also the tablets coated with Eudragit® RS30D and Eudragit® RL30D in the ratio 3:1 resulting in 3% coat load showed sustained release effect for 12 hr(as show in Fig.1). Both the formulations followed the higuchi model for dissolution. The dissolution of coated tablets were compared with the marketed preparation and shows that dissolution profile of the coated tablet were comparable with marketed preparation while the uncoated metformin tablet shows conventional dissolution profile.

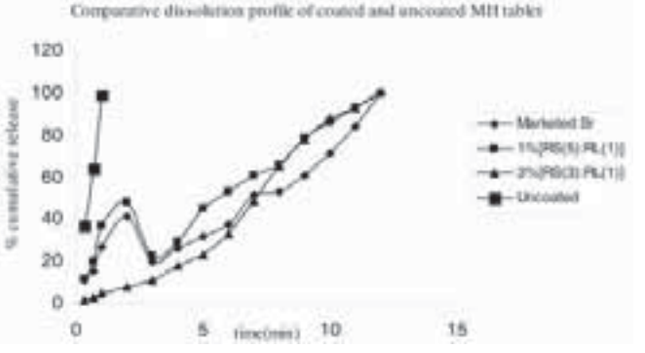

Figure1: Comparative dissolution profile of coated and uncoated metformin hydrochloride (MH) tablet.

CONCLUSION

Ammonio methacrylate copolymers, Eudragit® RL and RS are mainly used for sustained release preparation as film coat formulation. Coated tablets were shows excellent sustained release without burst effect. Also film coated tablets showed good stability upon aging under different storage conditions.

References

1. Aceves, J.M., Cruz, R., Hernander, C.E., 2000. Preparation and characterization of furosemide-Eudragit controlled release system. Int. J. Pharm. 195, 45–53.
2. Bodmeier, R., Guo, X., Sarabia, R.E., Skultety, P.F., 1996. The influence of buffer species on Deltiazem HCl release from beads coated with the aqueous cationic polymer dispersions, Eudragit RS, RL 30D. Pharm. Res. 13, 52–56.
3. Lehman, K., 1997. In: McGinity, J.W. (Ed.), Aqueous Polymeric Coatings for Pharmaceutical Dosage Forms, 2nd ed. Marcel Dekker, New York, pp. 101–176.
4. Okhamafe, A.O., York, P., 1983. Analysis of the permeation and mechanical characteristics of some aqueous-based film coating systems. J. Pharm. Pharmacol. 35, 409–415.
5. Lehman, K., 2001. Practical Course in Film Coating of Pharmaceutical Dosage Forms with Eudragit. Pharma Polymer. Darmstadt, pp. 8–10, 144–147.

PO119

HPMC matrix tablets swelling properties as a prediction tool for gliclazide release behavior by texture analysis

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INTRODUCTION

Hydroxypropyl methyl cellulose (HPMC)-based pharmaceutical matrix systems are widely used to extend the release of a broad range of pharmaceutically active materials. Swelling and release in matrices, viscosity and matrix porosity effects were mostly studied[1,2]. Different viscosity grades of HPMC have been proved to be suitable embedding materials for drug release by diffusion, erosion and particle erosion. However, the mechanism and kinetics of drug release are dependent on the solubility of the active moiety and the swelling and erosion properties of the polymer. The release rate changes are explained on the basis of the interaction between the gel and other matrix components in the presence of water[3]. Gel layer thickness has been considered as one of the key parameters affecting drug release from the swelling-controlled matrix system. Therefore, understanding of time-dependent polymer gel growth behaviour can be of a great assistance to formulation scientists in selecting the rational polymer. The aim of this study was to monitor water advancement, swelling process and gel growth by using texture analyzer and to investigate the kinetics of a low-soluble drug release from HPMC matrix tablets. Moreover, the influence of different preparation technology as well as particle size distribution on drug release rate was investigated. HPMC with nominal viscosities of 100 cP and of 4000 cP and gliclazide were used as model excipients/ drug combinations. Analysis of drug dissolution profiles in different pH media were made on the basis of Higuchi s model, power models and the Rosin Rammler Speding Weibull (RRSW) type equation. Gliclazide is widely used for the treatment of non-insulin dependent diabetes mellitus. It is a weak acid and has a very low solubility in gastric fluids and water, therefore, the release from sustained-release formulations could be pH-dependent.

EXPERIMENTAL METHODS

• *Sample preparation*

HPMC matrix tablets with gliclazide as a model drug were prepared by wet granulation or direct compression technology followed by compression. To minimize processing variables, all tablets were compressed under identical process conditions, on rotational tablet machine at a tablet weight of 160 mg according to routine tableting procedure. Tablet composition includes gliclazide, combination of methocel K100 LV CR, methocel K4M CR, dibasic calcium phosphate dihydrate, lactose monohydrate, colloidal silicon dioxide and magnesium stearate.

• *Swelling Properties*

A TA.XT2*plus* Texture Analyzer equipped with a 50 N load cell, and Texture Expert software (Texture Technologies Corp/Stable Micro Systems) was used to evaluate the swelling properties of the gliclazide formulations. Oval tablets that were 10 mm long, ~5 mm wide and 3 mm thick, and were free from physical imperfections were placed in the holder that was filled with the distillated water (23°C 1 °C). The flat aluminium (SMS P/25, Φ 25 mm) probe was lowered at pre-test speed of 1 mm/s, so that it would be in the contact with the tablet (trigger force 5 mN); the swelling force were recorded, and the data acquisition was terminated after 8.5 h. The measurement was repeated in triplicate.

• *In vitro dissolution method and pH values*

Dissolution tests were performed using dissolution apparatus 3 (reciprocating cylinder). The dipping rate was 15 dpm and temperature was 37 0.5 °C. The dissolution test was carried out in three stages: 1h in medium pH 1.2 (250 ml), 2 hours in medium pH 4.5 (250 ml) and 7 hours in medium pH 6.8 (250 ml). The samples were withdrawn at predetermined time points and assayed by HPLC method.

The HPLC instrument used was Agilent 1100, equipped with a diode array detector set at 235 nm. The column used was Zorbax Eclipse XDB-C8, 4.6 mm x 150 mm, 5 m (Agilent) with guard column. The mobile phase was acetonitrile + water + TEA + trifluoroacetic acid = 45 + 55 + 0.1 + 0.1 (v/v). The column was thermostated at 20°C, the flow

rate was 1.2 ml/min, the injection volume 20 l and the run time 10 minutes.

RESULTS AND DISCUSSION

a) Swelling behavior and method sensitivity

In order to determine accuracy and sensitivity of swelling force measurement method, samples with small differences in ratio of HPMC with nominal viscosities of 100 cP and 4000 cP were prepared. As expected, the samples with higher amount of Methocel K4M CR (4000 cP viscosity) showed higher swelling force.

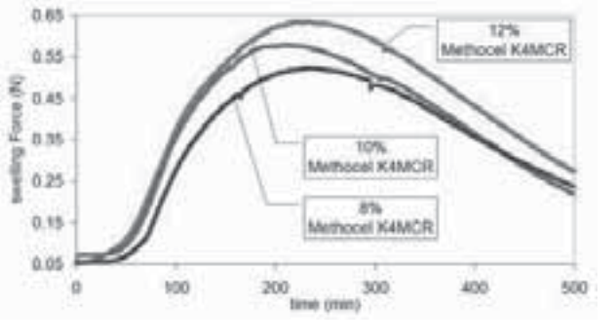

Figure 1: Swelling force of gliclazide tablets with Methocel K4M CR content of 12 % per tablet, 10% per tablet and 8 % per tablet.

b) Comparison of directly compressed and granulated formulations

The dissolution rate was found to be dependent on the preparation method, since the directly compressed (G-DC) formulation exhibits the significately higher dissolution rate. Figure 2 presents the Ritger and Peppas type model fit of gliclazide release from the tablets. It was observed that release from direct compressed formulation (G-DC) corresponds to non-Fickian diffusion mechanism and is pH independent.

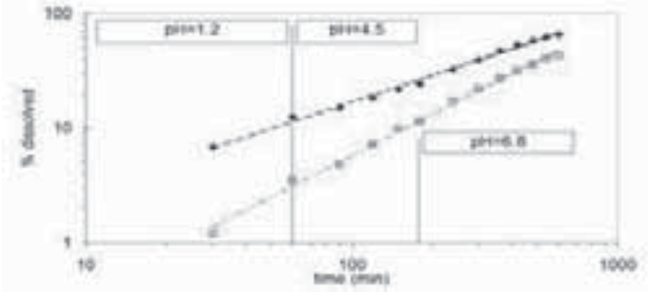

Figure 2: Ritger and Peppas type release model fit of gliclazide release from the tablets of directly compressed (G-DC) and granulated (G-WG) formulations.

c) The influence of the particle size distribution of granules on swelling behavior and release

Gliclazide release from the differently sized granules can be described by the Rosin Rammler Sperling Weibull (RRSW) type release model (Figure 3).

The formulation that has an increased portion of smaller size granules (Gl-G-R5) has a more exponentially shaped release than for the formulation that has an increased portion of larger granules (Gl-G-R2). The sample Gl-G-R5 also shows faster release. It can be concluded that controlled release can be achieved by controlling the particle size distribution (PSD) of granules.

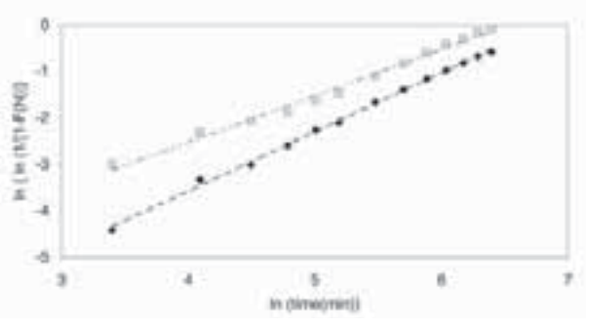

Figure 3: Fit of gliclazide release from the tablets of different size granules by the Rosin Rammler Sperling Weibull (RRSW) type release model; Gl-T-R2, Gl-T-R5.

CONCLUSION

The aim of this study was to monitor swelling process and gel growth by using texture analyzer and to investigate the kinetics of drug release from HPMC matrix tablets. The texture analyzer method for monitoring swelling process proven to be sensitive to small differencies in gel properties. Drug release mechanism is pH independent and was found to be a complex combination of diffusion, swelling and erosion. However, this study shows that the matrix swelling and gliclazide release is mainly achieved by diffusion process caused by the softening of the gel formed.

References

1. Feely, L.C. and Davis, S.S., 1988. *Int. J. Pharm.* **44** 1–3, pp. 131–139
2. R., Colombo, P., Massimo, G., Catellani, P.I. and Vitali, T., 1994. *Eur. J. Pharm. Sci.* **2** 3, pp. 213–219
3. S. and Ganderton, D., 1991. *Int. J. Pharm.* **70** 1–2, pp. 69–75

PO120

Swollen matrix tablet gel structure regulates the drug release rate

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INTRODUCTION

Hydrophilic polymers that can swell are the main matrix excipients for most of the modified-release tablet preparations. Among all of the semi-synthetic polymers, cellulose ether derivatives are the most frequently used polymers. However, polymers of natural origin are now coming to the fore of pharmaceutical research (1).

For hydrophilic polymers, it is generally accepted that, once in contact with water, they hydrate and swell, forming a gel layer that regulates the penetration of water into the tablet and the dissolution of the incorporated drug. The structure of the swollen polymer matrix gel is dependent mostly on the type of dissolution medium, polymer type and surface characteristics, according to its thermodynamic parameters (2). However, the release of the incorporated drug is not only dependent on its physicochemical characteristics; but is to a great deal, influenced by the gel layer structure (2, 3).

The aim of this study is focused on the relation between the structural characteristics of swollen tablets, made of some cellulose ether derivatives or xanthan as polymer of natural origin, and the release of pentoxifylline as a model drug. In elucidating the formation and properties of the gel layer during tablet swelling, the technique of nuclear magnetic resonance (MR) imaging was used. The MRI results, together with swelling data, could provide some answers involved in understanding the behaviour of drug release.

EXPERIMENTAL

Materials: The cellulose ether derivatives used were hydroxyethyl cellulose (HEC, Natrosol Aqualon; MW 1,200,000), hydroxypropyl cellulose (HPC, Klucel, Aqualon; MW 1,150,000), hydroxypropyl methyl cellulose (HPMC, Premium Methocel K4M, Colorcon; MW 95,000) and xanthan (XAN; Sigma-Aldrich Chemie; MW 2 10^6). Pentoxifylline, with MW 278.31 and solubility in water of 191 mg/ml, was supplied by Krka, d.d., Slovenia.

Preparation of matrix tablets: For MR imaging polymer powders were directly compressed to form tablets of hardness 100N 10 (VanKel VK 200, USA; hardness tester; n=6), m=500mg, 2r=12mm. During MRI experiments only one circular surface of the tablet was left uncovered for water penetration. For swelling studies similarly uncoated tablets were used, however, for drug release studies, 100mg of drug was homogeneously incorporated into 400mg tablets.

MR-imaging parameters: Sequential MR-images of matrix tablets during swelling in water were taken at room temperature, using a Bruker Biospec System (Bruker,G), equipped with a superconducting magnet (Oxford Instruments Ltd., UK) having a static magnetic field strength of 2.35T. The proton (^{1}H) NMR frequency of the spectrometer was 100 MHz. A standard spin-echo sequence was used, with a repetition time of 200ms, echo time of 1ms, and 4 averages to achieve a satisfactory signal-to-noise ratio. The total imaging time was 3 min 58 s. The field of view was approx. 5 cm, with in plane resolution of 200mm and the slice thickness of 3mm.

Swelling studies: These were performed in a purified water on a dissolution apparatus using the paddle method (Apparatus II, VK 7000, USA); V = 900ml, 50 rpm; T=37°C ± 0.5. After various times the partially hydrated tablets were carefully removed and weighted. The swelling degree was calculated as

$$\text{swelling degree (\%)} = \frac{m_t - m_r}{m_r} \times 100$$

m_t - mass of a hydrated tablet as function of swelling time (g); m_r - mass of remaining dry tablet after the swelling (g)

Drug release studies: These were performed under the same conditions as those for swelling studies. At predetermined time intervals, 10 ml samples were withdrawn and analysed UV-spectrophotometrically at 274 nm (HP diode array UV spectrophotometer, 8453, G).

RESULTS AND DISCUSSION

Gel layer characteristics of swollen polymer tablets are of crucial importance for the understanding of the drug release mechanisms and kinetics. The formation and growth of the hydrogel layer on the tablet surface is clearly seen on the MR images (Fig. 1). Moreover, the boundary between the dry core and the surrounding hydrogel, called the swelling front, and the boundary between the swollen polymer and the medium, called the erosion front, can be clearly distinguished. Both front positions are important in regulating the drug release rate.

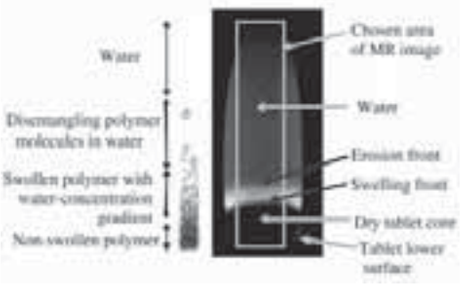

Figure 1: Schematic representation of the tablet swelling process, as examplified by the MR image area (HPC tablet after 9 hours of swelling).

From the MRI results, the gel layer thickness was determined in the following order: XAN > HEC > HPMC > HPC. The same order was proved also by the swelling degree data. The highest amount of water was incorporated into the XAN matrices (Fig. 2), followed by those of cellulose ether derivatives.

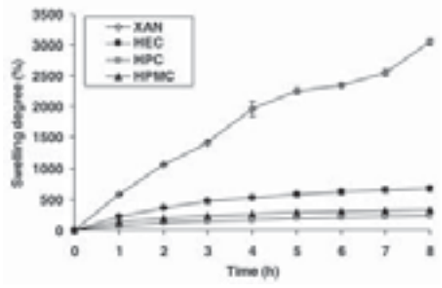

Figure 2: Swelling degree of diffrent polymer matrices as a function of time

Since XAN is an anionic polyelectrolyte with a semi-rigid chain structure, it is expected that the XAN matrix would result in a fundamentally different release behaviour than that for semi-synthetic polymers with flexible chains, like the cellulose ethers. This was proved by the drug release data (Fig. 3).

On the one hand, a thicker gel layer and more swollen structure of XAN resulted in a slowest drug release. On the contrary, for cellulose ether derivatives, a faster swelling and thicker gel layer resulted in a faster drug release. These differences arise from differently structured hydrogel layers as well as from a different arrangement of water molecules within. In water carboxylic groups of XAN are ionized and repel each other. XAN chains present many hydrophilic groups exposed to the water medium. The interactions between XAN and water molecules are favourable and the degree of swelling is thus high. On the other hand, hydroxyl groups of cellulose ether polymers are less swollen and interpolymer interactions are expressed to a larger extent, consequently relatively larger amounts of free water molecules are available among polymer chains, allowing a faster drug dissolution and diffusion out of the tablet.

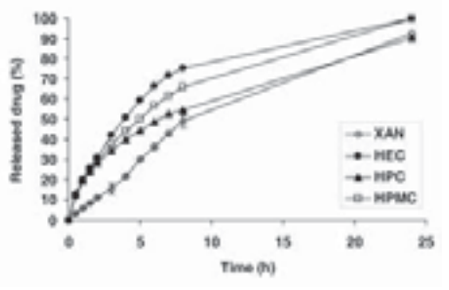

Figure 3: The release profiles of pentoxifylline in purified water from different matrix tablets

CONCLUSION

To understand the drug release behaviour from hydrophilic polymer matrices the interplay of different factors affecting the gel layer structure should be considered. Only a firm matrix structure that is accompanied by low swelling is not enough to guarantee a slow drug release.

References

1. Baumgartner S, Pavli M, Kristl J. Eur. J. Pharm. Biopharm. 2008; 69, 698
2. Baumgartner S, Lahajnar G. et all. Eur. J. Phar. Biopharm. 2005; 59, 299.
3. Fukuda M, Peppas NA, McGinity JW. Int. J. Pharm. 2006; 310, 90.

PO121

The influence of the formulation on the production and quality control of prolonged-release tablets with indapamid

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INTRODUCTION

The objective of this study was to formulate coated 1.5 mg indapamid tablets with prolonged release, in order to obtain a stable product, containing appropriate doses of active ingredient.

For this, indapamid was included in hydrophyllic matrices, using different types of polymers, in order to ensure a prolonged release, with values for the dissolved indapamid percentages and release profiles similar to those of the refference product, Tertensif SR 1,5mg, produced by Les Laboratoires Servier, France.

Indapamid is used in the treatment of hypertension, as a diuretic, in two different ocncentrations and in two dosage forms with distinct bio-pharmaceutic design, clinically argumented: immediate-release tablets (with 2.5 mg of active ingredient) and prolonged-release tablets (with a drug concentration of 1.5 mg). The prolonged-release product can be successfully used when an urgent diuretic effect is not required and when the risk of hydro-electrolythic alterations is high.

The formulation of prolonged-release indapamid tablets was based on o hydrophillic matrix. The product was manufactured by direct com-

pression, using two types of polymers: hydroxypropylcellulose (HPC) and hydroxypropylmethylcellulose), used in different concentrations: 17%, respectively 30%. It is quoted in the literature that a minimal concentration of 15 – 17 % of polymer is enough to generate a hydrophyllic matrix. Four formulations with 1.5 mg of indapamid were developed. (Table 1).

The purpose of the formulations was:

- to ensure an adequate density of the mix subjected to direct compression,
- to ensure an adequate flow of the mix by using a combination of lubricants,
- to ensure an optimal dissolution profile, the drug being relesed by diffusion and errosion (as gel-forming polymers we have used hydroxypropyl cellulose, respectively hydroxypropylmethyl cellulose),
- to produce cores with a low friability, so that they may be subjected to film-coating,
- to ensure the stability of the active ingredient was ensured by using a polymeric film which also acted as a barrier for light (containing titanium dioxyde as an opacifyer – E171).

MATERIALS AND METHODS

As materials we have used :

- Indapamid, powder
- Lactose monohydrate – Ludipress (BASF, Germany),
- Hydroxypropylmethylcellulose – Methocel K4M Preium EP (Colorcon, U.K.),
- Hydroxypropylcellulose – Klucel EXF (Aqualon Hercules, USA),
- Colloidal silicone dioxide – Aerosil 200 (Degussa ,Germany)
- Magnesia stearate (Faci Spa.,Italia)
- Corn stach, powder
- premix of excipients for tablet coating – Opadry® II 85F18378 White.

Table 1: Formulations of prolonged-release tablets with indapamid (mg/tab)

INGREDIENTS	F1	F2	F3	F4
Indapamid	1,5	1,5	1,5	1,5
Ludipress	111,4	111,5	111,4	111,5
Klucel EXF	30,6	54,0	-	-
MethocelK4 Premium CR	-	-	30,6	54
Luviskol K30	8,5	-	8,5	-
Corn starch	18,0	-	18,0	-
Aerosil 200	-	2,5	-	2,5
Carmellose sodium	2,0	-	2,0	-
Magnesia stearate	-	2,5	-	2,5
Opadry®II 85F18 white	8,0	8,0	8,0	8,0

The stages of the manufacture process were as follows:

- the preparation of the materials (drying, powdering, sieving),
- the preparation of the compression mix: indapamid, colloidal silicone dioxide and a part of the hydrophyllic polymer are homogenised for 30 minutes, the rest of the excipients is added (the remaining amount of polymer, lactose monohydrate, corn starch, magnesia stearate) and the homogenisation is continued for 30 minutes.
- the tableting was performed on a IMA KILIAN SYNTHESIS 300 rotating tableting press, with dye and punches with a diameter of 9 mm, lenticular, not inscribed).
- the resulting cores are loaded in the coatign drum and coated with the polymer [2]. The film-coated tablets are collected in stainless steel boxes, then packaged in blisters with transparent PVC / aluminium foils.

The tablets were tested immediately after manufacture and after 6 months, determining the organoleptic characteristics, weight uniformity, mechanical resistance, friability, indapamid content and the release characteristics of the tablets.

The equipment used for the quality control determination were:

- for the determination of the mechanical resistance: Erweka TBH 30 MB apparatus and the method described in the Eur. Ph. 5th edition;
- for the determination of friability: Vankel friabilator and the method described in the Eur. Ph. 5th edition;
- for the identification of indapamid: liquid chromatograph Agilent, by an HPLC method;
- the determination of the release of indapamid from the tablets: Hanson Research SR8 Plus dissolution station, according to Eur. Ph. 4 and the monography Indapamide Tablets from USP 28.

All the experimental formulations were compared to the reference product, according to the specifications described by the Romanian Pharmacopoeia Xth edition, the Eur. Ph. 5th edition, the U.S.P. 27th edition. [3]

RESULTS AND DISCUSSIONS

The resulting tablets have a smooth surface, white colour, round shape, a 9 mm diameter, 180 mg weight and a content of 1.5 mg indapamid / tablet. Based on the results, it can be stated that the formulations are adequate regarding the provisions of the Romanian Pharmacopoeia Xth edition and the Eur. Ph. 5th edition for weight uniformity, friability, mechanical resistance and disintegration.

The most relevant test for the prediction of the in vivo effect is the dissolution test. The results of this test indicate that the dissolution is influenced by the type and amount of polymer in the matrix. Good results are obtained for high concentrations of polymer. The data for the first three formulations F1 – F3 (with 17 %, respectively 30 % HPC and with 17 % HPMC), obtained after determining the dissolution profile of 6 tablets, have revealed a release of indapamid under the admissible limits.

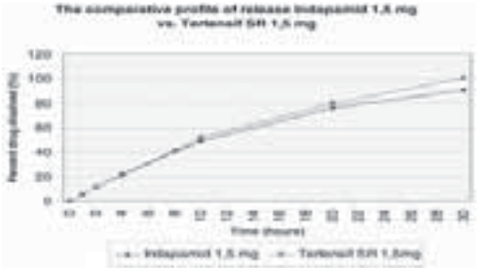

Figure 1: The comparative release profile of Indapamid 1,5mg – Tertensif SR 1,5mg

CONCLUSIONS

The limiting stage of the absorbtion process is the release of the drug from the dosage form. The tablets with 30 % hydroxypropylmethyl cellulose ensure a prolonged release, for over 30 hours, while those with only 17 % polymer are not suitable, manifesting a faster release of the ctive ingredient. In order to obtain the desired release profile, the cores had to be of 180 mg (the amount of hydroxypropylmethyl cellulose for the formation of a gel with the desired release properites was of 54.0 mg, 36 times bigger than the amount of drug).

References

1. Ansel C. H., Popovich N.G., Allen L.V. : Pharmaceutical dosage forms and drug delivery system, 6th ed., William & Wilkins, 1995
2. Lindsted B.: Release mechanisms of membrane coated depot formulations, industrial aspects of pharmaceutics, ed. by Sandell E., Swedish Pharmaceutic Press, stockholm, 1992.
3. European Pharmacopoeia

PO122

Influence of some formulation factors on the release of felodipine from prolonged release matrix tablets

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INTRODUCTION

The aim of this study was the preparation of prolonged release over 12 hours matrix type tablets containing felodipine. A reduced experimental design with 4 factors and 2 variables was used in order to observe the influence of the formulation variables on the characteristics of the tablets. The employed polymers in the formulations were: hydroxypropylmethylcellulose and polyethylene oxide [1, 2].

The objective of this study was to see the influence of diferent extended release polymers, different percents of polymers and the ratio between them on the release of felodipine from experimental prolonged release tablets.

MATERIALS AND METHODS

Materials. Polyethylene oxide (Polyox WSR Coagulant – Mw = 5,000,000 and Polyox WSR 1105 – Mw = 900,000, Union Carbide Europe), Hydroxypropyl methylcellulose (Methocel E4MCR – Viscosity = 3000 – 5600cP - 2% solution, and Methocel K100M – Viscosity of 80,000 – 120,000cP - 2% solution, Colorcon, UK), Lactose (DMV, Holland), Citric acid (Chemopar, Romania), Vitamin C (BASF, Germany), fumed silica - Aerosil (Degussa, Germany), Magnesium stearate (Merck, Germany), Felodipine (Everlight Chemical Industrial Corporation, Taiwan), sodium lauryl sulfate (Merck, Germany).

Experimental design. In order to see the influence of the formulation factors on the release of the felodipine a reduced experimental design with 4 factors and 2 variables was developed. The studied factors were the percent of hydrophilic polymers blend, the nature of hydrophilic polymers and the ratio between the polymers (Table I). In table II respectivly table III are presented the matrix of experimental design and the studied responses.

Table 1: Independent variables

Formulation variables	Symbol	Levels	
Percent of prolonged release polymers blend (%)	X1	30%	50%
Type of HPMC	X2	E4MCR	K100M
Type of Polyox	X3	Coag	1105
Ratio between prolonged release polymers (HPMC/Polyox)	X4	0.5	1

Tabel 2: The matrix of the experimental design

Exp. No.	**X1**	**X2**	**X3**	**X4**
1	30	E4MCR	WSR 1105	1
2	50	E4MCR	WSR 1105	1
3	50	E4MCR	WSR 1105	0.5
4	30	E4MCR	Coag	1
5	50	E4MCR	Coag	1
6	50	E4MCR	Coag	0.5
7	30	K100M	WSR 1105	1
8	50	K100M	WSR 1105	1
9	50	K100M	WSR 1105	0.5
10	30	K100M	Coag	1
11	50	K100M	Coag	1
12	50	K100M	Coag	0.5

Tablet preparation. The 8 mm diameter tablets were prepared by direct compression using the EK-0 Tablet press (Korsch, Germany). All the formulations contained 10 mg felodipine/tablet and the weight of the tablets was established at 210 mg. Lactose was used as filler, the cit-

ric acid, vitamine C as anti-oxidants and magnesium stearate, Aerosil as lubricants.

Table 3: Responses

No.	Response	Symbol
1	% released at 0.5 hours	Y1
2	% released at 1 hour	Y2
3	% released at 1.5 hours	Y3
4	% released at 2 hours	Y4
5	% released at 3 hours	Y5
6	% released at 4 hours	Y6
7	% released at 5 hours	Y7
8	% released at 6 hours	Y8
9	% released at 8 hours	Y9
10	% released at 10 hours	Y10

In vitro evaluation. The *in vitro* dissolution studies were performed according to an adapted method from the USP 27 "Felodipine Extended - Release Tablets" [3]. The PharmaTest PT-DT7 device was used. This was equipped with the no. 1 apparatus (basket) at 50 rpm rotation speed. The dissolution media employed was 500 ml of phosphate buffer with pH=6.5 and 1% sodium lauryl sulfate at 37 0.5º. Samples were collected at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8 and 10 hours. The samples solutions were analyzed at 240 m using a HPLC Agillent 1100 series equipped with a Zorbax SB-C18, 5 m x 4.6 x 250 chromatographic column, mobile phase: acetonitrile: phosphoric acid 0.1% in water = 75:25 at a flow of 1.5 ml/minute and 3 minutes retention time.

RESULTS AND DISCUSSIONS

Experimental design analysis. The data fit was done using the Partial Least Square method. In all cases the data fit was good or very good.

The study of in vitro release of felodipine. Studying the release profiles of the experimental formulations (Fig. 1.) we had observed that the felodipine was slowly released over 12 hours; between 40% - 75% in case of formulation 5, 8, and 9, 80% formulations 2, 3, 6 and 7 and completely released from the formulations 1, 4, 10, 11 and 12.

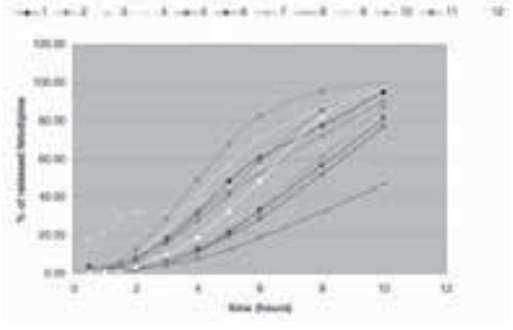

Figure 1: Release profiles from the studied extended release tablets

Influence of the formulation factors on the tablet characteristics. The influence of the formulation factors on the release of felodipine at different time intervals are shown in fig. 2.

The most important variable is the percent of extended release polymers blend (X1); by increasing the percent form 30 to 50% a reduced release of the drug in all formulations was observed at all the studied time intervals except the first 2 hours of release (Fig.1.). The second important variable is the ratio between the extended polymers (X4) as shown in fig. 2; by increasing the ratio of HPMC in the formulas from 0.5 to 1 the % of felodipine released in the first 4 hours of disolution was diminished (Fig.2. Y_1, Y_3). In the first hours of the release the presence of HPMC E4MCR (X2) increased the drug release (Fig.2 Y_1, Y_3).

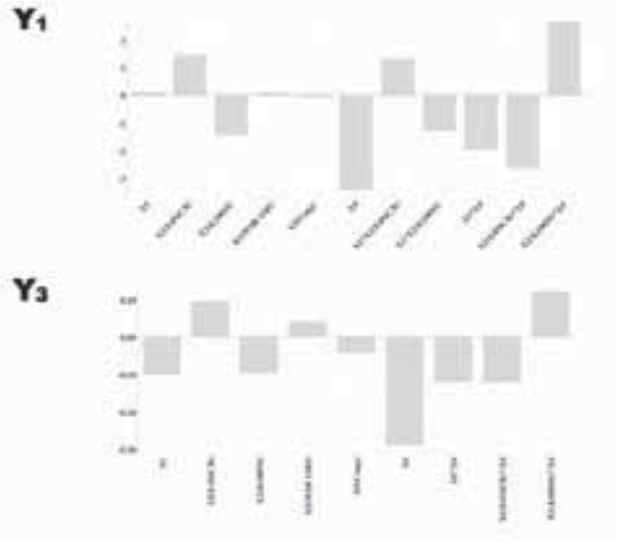

Figure 2: Influence of the studied variables on the % of released felodipine at some release times

CONCLUSIONS

A prolonged release profile of felodipine over 12 hours from the experimental tablets was obtained using 50% polymers blend or by increasing the ratio of HPMC in the polymers blend.

References

1. Vueba M.L., Batista de Carvalho L.A.E., Veiga F., Sousa J.J., Pina M.E., Influence of cellulose ether polymers on ketoprofen release from hydrophilic matrix tablets, European Journal of Pharmaceutics and Biopharmaceutics 58, 51–59, 2004
2. El-Malah Y., Nazzal S., Hydrophilic matrices: Application of Placket–Burman screening design to model the effect of POLYOX–carbopol blends on drug release, Int. J. Pharm., 309 (1-2), 163-170, 2006
3. * * * United States Pharmacopeia, 27th Edition, "Felodipine Extended - Release Tablets", 778-781, 2004

PO123

Sustained release of caffeine from inert polymers – a percolation theory approach

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INTRODUCTION

Percolation theory has had many applications in pharmaceutical technology all of them being in the purpose of making robust formulations. Sustained drug release is always a challenge and percolation analysis provides powerful tools for solving formulation issues. The main postulate of percolation theory, shown in the Eq. 1, is that system s property X follows a scaling law:

$$X = S(p - p_c)^q \quad (1)$$

where S and q are constants, p denotes occupation probability with p_c being a percolation threshold. Percolation threshold describes a state of a system when it has just seized a connected network of its constituent. At this point system s properties suddenly change and it should be carefully avoided.

In the case of matrix tablets designed for sustained drug release one of the biggest obstacles is high solubility of the drug molecule. Inert polymeric materials have been investigated in order to determine the possibility for their use in sustained drug release. Percolation theory enables us to find exact amount of matrix forming material for slowing the drug release rate and also to predict system behaviour. Caffeine was chosen as a model substance with high water – solubility; methacrilate copolymer Eudragit® E PO and glycerol behenate ester base Compritol® 888 ATO were chosen as inert matrix forming materials.

MATERIALS AND METHODS

The following materials were obtained from suppliers: Caffeine, Compritol®888 ATO (Gattefossé GmbH, Weil am Rhein, Germany) and Eudragit® E PO (Röhm GmbH, Darmstadt, Germany). Caffeine – Eudragit® E PO matrices were prepared by compressing a homogeneous mixture of powders with an excenter tablet press (Eko Korsch, Germany). Caffeine - Comprtiol®888 ATO matrices were prepared via hot – melt granulation process. Prior to compressing Compritol®888 ATO was melted on a water bath with continuous stirring maintaining the temperature approximately at 70°C. When the temperature was around the congealing point the molten mass was screened through a 1 mm sieve.

Caffeine s release was studied in dissolution apparatus (Erweka DT6, Hausenstamm, Germany). using the rotating paddle method (75 rpm) for Eudragit® matrices and rotating basket method (50rpm) for Compritol® matrices. 900 ml of phosphate buffer, pH=6,8 (USP 28) was used as dissolution media. Dissolution tests were conducted for 8 hours. The amount of caffeine was determined using UV spectrophotometer (λ=272nm).

Table 1: Composition of Caffeine – polymer matrices

Batch	% w/w Caffeine	% w/w Eudragit®	% w/w Compritol®	%v/v Caffeine + initial porosity*
F1	50	50	-	36,12
F2	45	55	-	29,54
F3	42,5	57,5	-	24,01
F4	40	60	-	19,75
F5	35	65	-	10,20
F6	30	70	-	6,03
F7	95	-	5	94,15
F8	92,5	-	7,5	90,86
F9	90	-	10	87,97
F10	87,5	-	12,5	84,53
F11	85	-	15	80,41
F12	80	-	20	75,03
F13	70	-	30	61,20
F14	60	-	40	50,73

* %v/v Caffeine + initial porosity ε was calculated based on tablet's geometry and density of both caffeine and polymer

Linear regression analysis has been employed in order to characterise caffeine's release. Being inert and non-water soluble matrix forming materials, Eudragit® and Compritol® enable caffeine's release only to be diffusion controlled process. Higuchi's equation was used to fit the experimental data:

$$Q = k_h t^{1/2} \quad (2)$$

where Q is the fraction of caffeine released at time t and k_h is simply the Higuchi rate constant. Furhter modeling was used to estimate the percolation threshold as well as to solve Eq.1 which makes it possible to predict system's behaviour and design robust formulations.

RESULTS AND DISCUSSION

Figure 1 demonstrates the release profiles obtained from caffeine – polymer matrices.

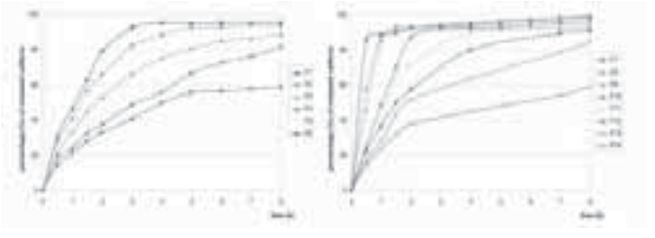

Figure 1: Release of caffeine from batches F1 – F14

Based on fiting the caffeine s release data in Eq.2 percolation threshold was determined:

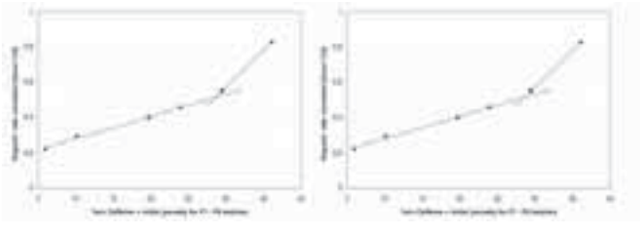

Figure 2: Determination of percolation threshold for batches F1 – F14

Percolation threshold ε_c was found to be at 28,66 and 82,94 %v/v caffeine + initial porosity for matrices with Eudragit® and Compritol® respectively. It can clearly be seen from the Fig.1 that only when the certain amount of polymer is reached the release of caffeine slows down. This is because polymer's chains form an infinite network entrapping caffeine s molecules. When the concentration of polymer exceeds the critical point at percolation threshold there are some clusters of caffeine's molecules that never get to be released. This is why it is necessary to be able to predict the drug's release rate because even though increasing the ratio of the polymer slows the drug release it also decreases the amount of drug released. Figures 3 and 4 show the relationship between %w/w of polymer, %v/v of caffeine + initial porosity and Higuchi s constant k_h. From these relationships models have been developed and are shown here in Equations 3 and 4.

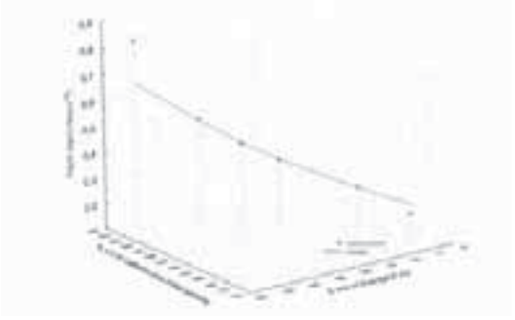

Figure 3: Modeling of the caffeine's diffusion rate from Eudragit® matrices

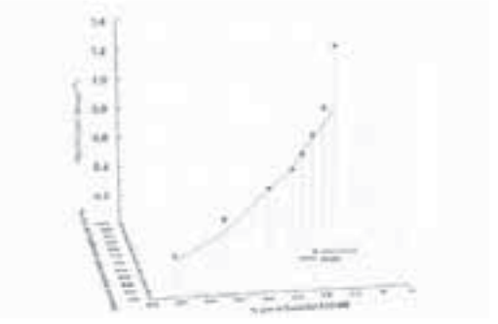

Figure 4: Modeling of the caffeine s diffusion rate from Compritol® matrices

The following models were obtained:

$$k_h = 0{,}2149 \exp[0{,}0315(\varepsilon - \varepsilon_c)] \quad (3)$$

for matrices with Eudragit®, and

$$k_h = 0{,}0347 \exp[0{,}033(\varepsilon - \varepsilon_c)] \quad (4)$$

for matrices with Compritol® with correlation coefficients for both models being $r^2=1$. Models enable us to predict drug release rate for known amount of drug + initial drug's porosity ε.

As can be seen from Figures 3 and 4 models are valid close to the percolation threshold and even wider than anticipated.

References

1. J. Hamdani et al. / International Journal of Pharmaceutics 260 (2003) 47–57
2. H. Leuenberger et al. / International Journal of Pharmaceutics 115 (1995) 217-224

PO124

Mathematical modelling of drug release from drug: Polyox WSR Coagulant matrix

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INTRODUCTION

The modern tendency in pharmacotherapy is the application of the preparations with modified drug release, with attention to development of matrix configurations due to their numerous advantages: producing involves simple technological operations (direct compression), they can also contain drug with high molecular weight, due to the fact that the drug is dispersed in the system there is a slight possibility of „leaking". The occurring problems in matrix producing process are mostly connected to polymers used in the process, because they sometimes do not have good flowability, which impedes the direct compression and bad compressibility results in tablets of small hardness which effects the drug release profile. Because of this, characterization of new poly-

mer used in the process of producing matrix systems is being worked on. New generation polymers, such as Sentry® Polyox® show numerous favourable characteristics which make them candidates for wide application in the process of producing matrix tablets with modified release. Sentry® Polyox® are non-ion, hydrophilic, polyethylene oxides polymers with high molecular weight.

In this study mechanism of diclofenac-sodium release from the matrices produced from Polyox® WSR Coagulant were investigated by applying the mathematical models and modelling the processes of polymer swelling.

Polyox® WSR Coagulant (Mr=5x10^6) belongs to the PEO group of high molecular weight that have potentially wide application in producing matrix tablets because of their non-toxicity, high water-solubility and swellability.

There is very little written data on application of these PEOs, especially for the polymers with Mr bigger than 1x10^6 .

MATERIALS

Sentry ®Polyox ® WSR Coagulant-LEO NF Grade (*Dow Chemical Company, Charlston, USA)* was used as a polymer. It is a white dusty substance, molecular weight of 5.000.000 and 1% solution has the viscosity of 5.500-7.500 cP (25°C).

The manufacturer claims that there isn't an interaction between the polymers and active substance due to its non-ion nature of polymers. Research showed that the polymers have good lubricant and bonding ability.

Diclofenac-sodium that meets the requirements of Ph.Eur VI was used as a model substance.

METHODS

• Preparation of comprimates

Drug – containing matrices were prepared by compressing a homogeneous mixture of the drug and polymer powders with an excenter tablet press (*Eko Korsch, Germany)*. Table 1 shows the composition of the studied batches (F1-F5).

The tablets' weight was kept constant at 450mg and a diameter of cylindrical tablets was 11mm

Table 1: Composition of the Drug-Polyox WSR Coagulant matrices

	F1	F2	F3	F4	F5
Diklofenac-Na	96%	95%	90%	70%	50%
Polyox® WSR Coagulant	4%	5%	10%	30%	50%

• Drug release study

Diclofenac-sodium release from the produced comprimates (F1, F2, F3, F5) was investigated using the rotating paddle apparatus (*Erweka* DT6, Hausenstamm, Germany) at stirring speed 50 rpm. Phosphate buffer pH 6,8 (USP 31) was used as a medium. Volume of the medium was 900 ml, Diclofenac-sodium release was conducted for 8 hours. Within the first 3 hours samples were taken every half an hour, and then after every hour. The amount of diclofenac sodium was determined using UV-VIS spectrophotometer (λ = 275nm). Cumulative percentage of drug release rate was calculated and used in data analysis.

Profiles of the drug release rate were analysed by applying various mathematical models to show which type of kinetics best describes the examined profile. Following models were used:

Zero order kinetics	$M = M_0 - k_0t$
First order kinetics	$lnM = lnM_0 - k_1t$
Higuchi diffusion model	$Q = k\sqrt{t}$
Krosmayer-Peppas model	$Q = kt^n$

Where:

M_0 – amount of the undissolved substance at the beginning of dissolving process (t=0)

M – amount of undissolved substance after a period t

Q – amount of dissolved substance after a period t ($Q=M_0-M$)

k_0, k – the appropriate constant of dissolving velocity

n – diffusion coefficient

• Swelling study

The swelling process of polymers, was evaluated by dipping the comprimates into 20ml of medium (phosphate buffer pH=6,8) and measuring the mass change of the comprimates for 8 hours. The analysis of swelling kinetics was evaluated using davidson-Peppas model:

$W = kt^n$

Where W is water mass which comprimate imbibes during the period t.

RESULTS AND DISCUSSION

Release bahaviors of diclofenac sodium from the comprimates are shown in Figure 1.

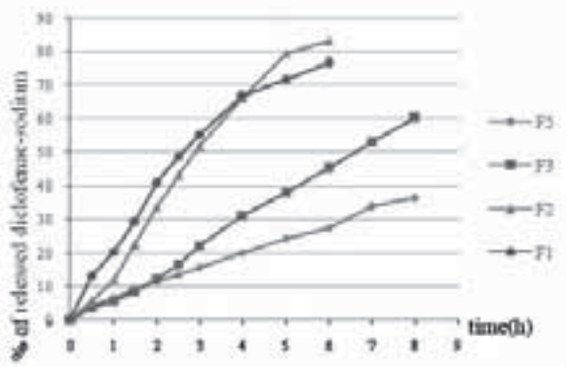

Figure 1: Diclofenac sodium release from comprimates F1-F5

Sustained drug release from all prepared comprimates was attained. When drug release from formulations F1 and F2 (with 4% and 5% of polymer) was monitored, high values of Standard deviation was obtained, indicated variations in percent of drug release within the same bach. That's because the robusty of the formulation could not be maintained – even small drug release characteristics changes in the concentration would lead to changes in the drug release characteristics.

From the release profiles and their fitting in the mathematical models it was concluded that Krosmayer-Peppas's equation is the most appropriate for describing drug release from hydrophilic matrices (the highest values for correlation coefficient r^2). The results obtained by fitting the experimental data in mathematical models are given in Table 2.

Table 2: Values of diffusion exponent and the diffusion constant

	n	logk
F1	0,75	1,35
F2	1,14	1,12
F3	1,09	0,80
F5	0,78	0,84

Krosmayer-Peppas model $Q = kt^n$

$\log Q = \log k + n \log t$

The value of diffusion exponent **n**>0,75 shows that the release mechanism is the combination of diffusion and erosion , which is the consequence of the quality of the used polymer. These hydrophillic polymers swell in contact with water and form gel-layer around the tablet. Gel-layer is complex and represents the diffusion barrier for further water entering within the matrix, and can modulate drug release rate.

The substances dissolved in water leave the systems by diffusion and dissolving in the gel-coat, while the undissolved are probably released during the gel erosion. When the release mechanism includes both swelling of the polymers and the erosion of the gel-coat, the matrix system can allow the release of the drug with the zero order kinetics.

In this case the release of the diclofenac-sodium happened with the zero order kinetics, when 10% of polymers was used, which is indicated by the coefficient of the correlation r^2=0,996.

Swelling of matrices was more pronounced in the case of the comprimates with a greater percent of polymers, which was expected because of the molecular weight of polymers.

The swelling kinetics was well fitted using Davidson-Peppas model: $W=kt^n$

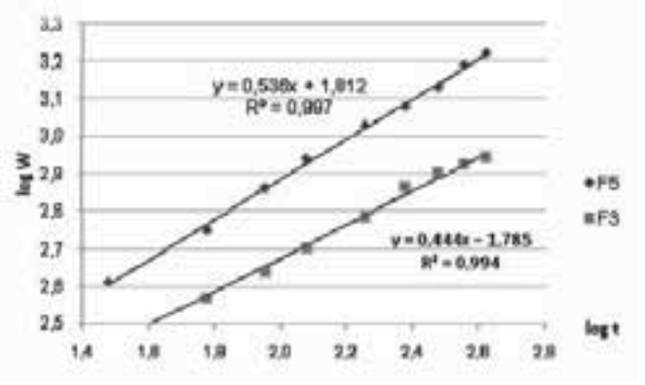

Figure 2: Swelling kinetics of polymer in F3 and F5

CONCLUSION

The given results indicate that applied polymer can be successfully employed in the formulation of the matrix tablets with controlled release of drugs.

References

1. L. Maggi, L. Segale, M. L. Torre, E. Ochoa Machiste and U. Conte: Biomaterials 23 (2002): 1113-1119
2. Ning Wu, Li-Shan Wang, Darren Cherng-Wen Tan, Shabbir M. Moochhala and Yi-Yan Yang**:** Journal of Controlled Release 102 (2005):569-581
3. Sentry® Polyox®-Products Regulatory Data Sheet,The Dow Chemical Company, US

PO125

The investigation of matrix tablets based on carrageenans for sustained release of doxazosin mesylate

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INTRODUCTION

Carrageenans are biopolymers which are mainly found in the cell walls of red seaweeds (*Rhodophyceae*). They are biocompatible, nontoxic and usually at low price, therefore they are interesting materials in pharmaceutical drug delivery design. Carrageenans are high molecular polysaccharides, which consist of Na^+, K^+, Ca^{2+} and Mg^{2+} sulfate esters of galactose and 3,6-anhydrogalactose copolymers (1). Three types of carragenans are used in pharmaceutical delivery systems, namely -ι -κ and -λ carragenan, which predominantly differ in amount of sulfate groups which give rise to their heterogeneous behaviour. The amount of sulfate groups is as follows: λ > ι > κ . The property of carrageenans to form stable hydrogels allows us to use them in the design of prolonged release matrix tablets (2).

Doxazosin mesylate is a quinazoline antihypertensive, a selective α_1-antagonist. According to it's properties – good bioavailability (65%), long plasma half-life (22 hours) and duration of action (18-36 hours) – achieving sustained drug release is desirable (3).

The aim of our work was to develop biopolymer matrix tablets for sustained release of doxazosin mesylate based on three different kinds of carrageenans (ι , κ , λ) as matrix formers. Drug release from obtained matrices was investigated together with polymer swelling and erosion, which are responsible for hydrogel formation in order to precisely evaluate characteristics of these matrices.

EXPERIMENTAL

Materials: ι, κ, and λ carrageenans (FMC Biopolymers, USA), active substance doxazosin mesylate (DM) (MM = 547,58 g/mol, supplied by Krka, d.d, Slovenia). *Preparation of matrix tablets:* carrageenans and active substance were homogeneously mixed. Tablets (m=300mg, 2r=10mm, hardness 50-100N, dose of DM=8mg) were prepared by direct compression (Kilian, USA). For the swelling and erosion determinations carrageenan polymers without doxazosin mesylate were compressed to matrices using the same procedure.

Drug-release studies were performed on a dissolution apparatus using the basket method (Apparatus I, VanKel, VK 7000, USA); basket speed=150 rpm, T=37 0.5 °C. The dissolution medium: 900 ml of either phosphate buffer of pH 7 with sodium lauryl sulphate (SLS) or phosphate buffer of pH 7 with SLS and NaCl. The concentration of sodium lauryl sulfate was 5 g/L and of NaCl 2 g/L. Analyses were performed UV-spectrophotometrically at 249 nm (HP 8453, GER). *The swelling and erosion studies* were performed in 900 ml phosphate buffer of pH 7 with SLS, without or with NaCl, using a dissolution apparatus equipped with paddles (USP Apparatus II, VanKel, VK 7000, USA). The paddle speed=50 rpm. After 1, 3, 5 and 8 h the partially hydrated matrices were carefully removed and the tablets were lightly dried with tissue paper to remove excess surface water prior to being weighed. After determination of the weight of the hydrated matrices, they were dried at 70 °C in a vacuum oven for 2 days, before determination of the remaining dry weight. The swelling degree and the percentage of erosion were calculated.

RESULTS AND DISCUSSION

Comparison of the results of drug release, erosion and swelling resulted in the following conclusions. Drug release from kappa carrageenan matrix is the fastest (Fig. 1) which correlates well with erosion (Figs. 2). Namely drug release is finished in concordance with erosion in about 8 hours. The same was found for lambda and iota matrix, which also have almost identical drug release and erosion profiles for 10 hours (Figs. 1, 2).

Swelling studies of carrageenans (Fig. 3) indicate that water uptake is relatively fast and high overall which means that a hydrogel around dry polymer matrix core is formed rapidly. Evident differences between different carrageenan types were observed.

The swelling studies revealed that swelling correlates well with the number of sulfate groups on polymers, the higher the number the faster and higher the swelling (lambda > iota > kappa). The result of abundant swelling of lambda and iota carrageenan is slower drug release comparing with less swollen kappa matrices. Interestingly although lambda and iota swell at least two fold of a magnitude more than kappa carrageenan, their erosion is evidently lower which leads us to assumption that hydrogels of lambda and iota carrageenan are stronger. Firmer hydrogel structure of the swollen matrix tablet enables slower drug release.

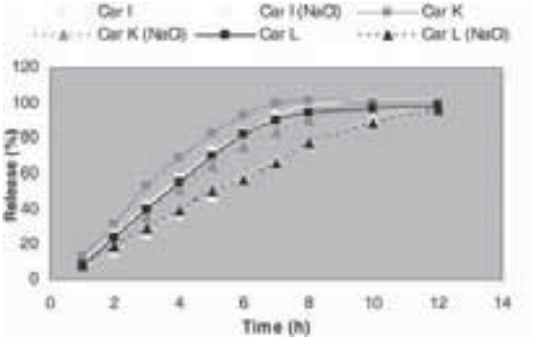

Figure 1: Drug release profiles of doxazosin mesylate (DM) from different carrageenan matrices in media with and withouth NaCl. K represents kappa, I iota and L lambda carrageenan (Car). The data are means SD of 6 measurements.

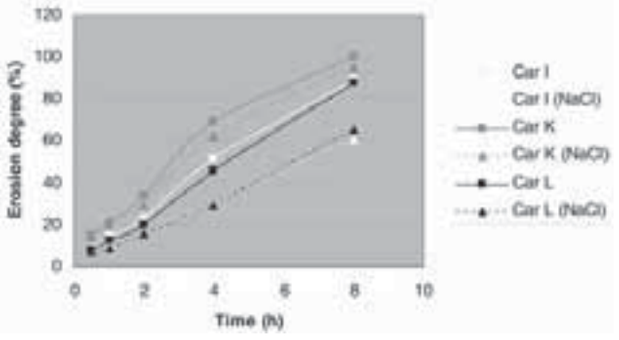

Figure 2: The erosion of different carrageenan matrices in media with and withouth NaCl. K represents kappa, I iota and L lambda carrageenan (Car). The data are means SD of 6 measurements.

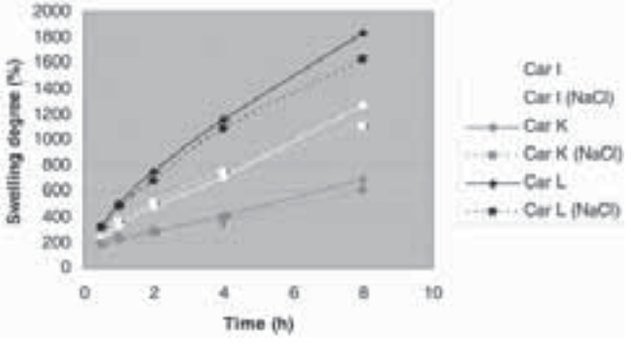

Figure 3: The swelling of different carrageenan matrices in media with and withouth NaCl. K represents kappa, I iota and L lambda carrageenan (Car). The data are means SD of 6 measurements.

In media with NaCl slower drug release, swelling and erosion for all carrageenan matrices were observed. Probably this was due to counterion condensation as proposed by theory of Manning (4), leading into the charge screening effect of the sulphate groups on the carrageenan moieties.

Also in case of presence of NaCl correlation of drug release and polymer erosion was obtained.

CONCLUSIONS

The results of our studies indicate that carrageenans can be used as matrix formers to sustain the drug release up to 10 hours depending on the type. Drug release in media with higher ionic strength is additionally prolonged up to 12 hours. Both swelling and erosion of different carrageenan matrix tablets are important in prolongation of drug release but it seems that polymer erosion is a dominant parameter since a good correlation between erosion and drug release was observed.

References

1. Rowe RC, Sheskey PJ, Weller PJ. Handbook of pharmaceutical excipients 4th ed. Pharmaceutical Press, 2003: 101-103.
2. Bhardway TR, Kanwar M, Lal R, Gupta A. Natural gums and modified natural gums as sustained-release carriers. Drug Dev and Ind Pharm 26 (2000) 1025-1038.
3. Williams DA, Lemke TL. Foye s principles of medicinal chemistry 5th ed. Lippincott Williams&Wilkins, 2002: 552-557.
4. Rivas BL, Moreno-Villoslada I. Evaluation of the counter-ion condensation theory from the metal ion distributions obtained by ultrafiltration of a system poly(sodium 4-styrenesulfonate)/Cd^{2+}/Na^{+}. J Phys Chem B 102 (1998) 11024-11028.

PO126

The influence of copolymeric chain microstructure on cyclosporine A and rapamycine (sirolimus) long-term release from p(L-LA-co-TMC) matrices

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INTRODUCTION

Biodegradable polymers obtained from lactide (L-LA) and trimethylene carbonate (TMC) are interesting materials for application in medical and pharmaceutical fields. P(TMC) characterizes surface erosion, that allows to obtain zero order kinetics of released drug as well as protection of labile drug molecules. Furthermore, acidic products are not released from these kind of polymer after degradation process [1,2]. These features are especially interesting for using homo- and copolymers of TMC (e.g. with L-lactide) as drug carriers and develeoping alternative delivery systems of agents that available dosage forms cause a lot of side effects, as Cyclosporine A (CyA) and Rapamycine (Sirolimus). There are attempts to obtain different kinds of polymeric carriers with CyA and Rapamycine [3,4], however none of them provide prolonged delivery. The aim of this study was to analyze the influence of copolymer chain microstructure of CyA and Rapamycine as the parameter that can be used for controlling and modifying the amount of released drug.

MATERIALS AND METHODS

Five kinds of poly(L-lactide-co-TMC) were used to prepare matrices containing 10-weight-% of Cyclosporine A and Rapamycine (Sirolimus) (LC laboratories): 1). (P(LLA70%-TMC-30%)); 2). (P(LLA73%-TMC27%)) - semiblock; 3). (P(LLA72%-TMC28%)) - random; 4). (P(LLA44%-TMC56%)); 5). (P(LLA70%-TMC30%))-PEG37%. Characteristic of copolymers microstructure during degradation process was conducted based on the parameters determined from ^{1}H and ^{13}C NMR spectra: the percentage content of copolymer units, the average length of the blocks, and randomization ratio, according to the equations presented in literature [5].

The ^{1}H and ^{13}C NMR spectra of copolymers were recorded on a AVANCE II Ultra Shield Plus, Bruker 600 MHz. $CDCl_3$ was used as a solvent. The molecular weight was determined by GPC (Physics SP 8800 chromatograph), with chloroform as eluent. The amount of released drug was determined by means of UV-vis spectrocopy (Spektrofotometr V-570, UV-VIS-NIR-JASCO). The 1,2cm diameter matrices were prepared from solution of each kind of copolymer in methylene chloride with 10 weight-% of one of two studied drugs.

RESULTS AND DISCUSSION

In the first stage of experiment, only Copolymer 1 and 2 were analyzed to confirm enough slow degradation process to provide long-term delivery for CyA and Rapamycine. The amount of released CyA during 689 days didn't exeed 48% (36,2% from Cop 1 and 47,3% from Cop 2) and percentage amount of released Rapamycine in the same time period was even lower (11,1% from Cop 1 and 13,6% from Cop 2) (Fig. 1,2). Regular release profile was observed for Cop 1, which characterized small changes of copolymer chain randomization during degradation process. Based on these results, further analysis were performed, comparing five kinds of copolymers obtained from the same kind of comonomers, but with their different percentage amount, and various chain microstructure. For Copolymer 1 and 2 semiblock structure was determined, whereas Copolymers 3-5 were random. Characterization of all copolymers used to prepare matrices with drug is presented in Table 1. Comparison between amount of released drug is shown in Fig. 1. For Cop 2 and Cop 3, having similar percentage comonomer ratio (73:27 and 72:28, respectively), differences in drug release profiles (Fig. 3), determined by differences in microstructure of copolymer were noticed. The release process was much more even for

Table 1: Characteristic of microstructure of poly(L-lactide-co-TMC) matrices containing CyA or Rapamycine (Mn- number-average molecular mass; l_{LL}, l_T-the average length of lactidyl and carbonate sequences; R – randomization ratio, T_{II} – transesterification ratio, Tg-glass transition temperature

Nr.	Kind of copolymer	Mn	The average length of the blocks	R	T_{II}	Tg
Copolymer 1	poly(L-lactide-co-TMC) (30:70)	17.500	l_{LL} = 4.11 l_T = 1.52	0.57	0.63	8.0
Copolymer 2	poly(L-lactide-co-TMC) (72:28)	36.500	l_{LL} = 6.28 l_T = 2.44	0.5	2.78	42.3
Copolymer 3	poly(L-lactide-co-TMC) (72:28)	86.700	l_{LL} = 3.90 l_T = 1.55	0.85	no data	42.3
Copolymer 4	poly(L-lactide-co-TMC) (44:56)	26.700	l_{LL} = 1.51 l_T = 2.05	0.82	2.05	22.6
Copolymer 5	poly(L-lactide-co-TMC) (70:30) 37% PEG	30.000	l_{LL} = 4.21 l_T = 1.64	0.73	no data	49.9

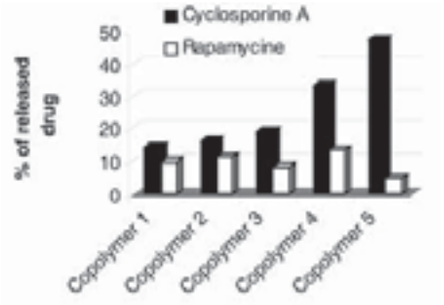

Figure 1: The amount of released drugs during 227 days from five kinds of poly(L-lactide-co-trimethyene carbonate) matrices.

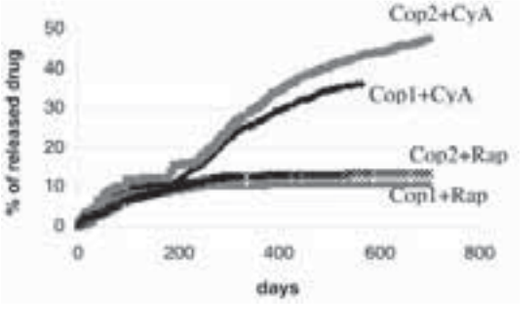

Figure 2: Cumulative release profile of Cyclosporine A and Rapamycine from Copolymer 1 and 2.

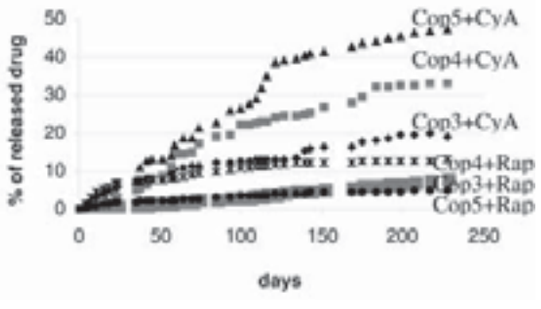

Figure 3: Cumulative release profile of Cyclosporine A and Rapamycine from Copolymer 3 and 4.

matrices prepared from Cop 3 (especially in case of Rapamycine), because of more regular degradation process, as a result of shorter the average length of the sequences and higher randomization ratio. The biggest differences between the amount of released drug (the highest amount of CyA and the lowest amount of Rapamycine) were determined for Cop 5 (with 37% of PEG).

CONCLUSION

All of the studied copolymers provide long-term relaese, appropriate for immunosuppressive drugs. However, close correlation between release profile and copolymeric matrice degradation, determined by microstructure of copolymer, was determined. Degradation process proceeds differently for three kinds of studied matrices, prepared from the same kind of copolymer, but containing CyA, Rapamycine or containing no drug. Moreover, different release profiles were observed not only for matrices with various chain microstructure, but also for each of two drugs.

ACKNOWLEDGEMENT

This study was financially supported by the Polish Ministry of Science and Higher Education (Grant No: NN 405 3201 33)

References

1. Nair LS, Laurecin CT. Biodegradable polymers as biomaterials. Prog. Polym. Sci; 32: 762 – 798 (2007)
2. Zhang Z, Grijpma DW, Fejen J. Creep-resistance porous structures based on stereo-complex forming triblock copolymers of 1,3 trimethylene carbonate and lactides. Journal of Materials Science: Materials in Medicine; 15: 381 – 385 (2004)
3. Gref R, Quellec P, Sanchez A, et al., European Journal of Pharmaceutics and Biopharmaceuticals 51: 111-118 (2001)
4. Alexis F, Venkatraman S, Rath SK, et al., Journal of Controlled Release 98: 67-74 (2004)
5. Dobrzynski P, Kasperczyk J. Synthesis of biodegradable copolymers wit h low-toxicity zirconium compounds. V. Multiblock and random copolymers of L-lactide with trimethylene carbonate obtained in copolymerizations initiated with zirconium (IV) acetylacetonate. Journal of Polym Sci Part A: Polym Chem. 44: 3184-3201 (2006)

PO127

Development of an antibiotic containing drug delivery system for surgical application

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INTRODUCTION

The orthopedic surgery, that has the aim of substitution of bone joint, is now the leading therapeutical solution for osteoarthrosis. The implants, that are in the body for long time, may cause complications (technical, biological). In revision surgery, when the implant is renewed, different bone supplement methods are available. The most important is the use of bone grafts [1, 2]. The implanted bone does not have own circulation until osteointegration, when the implanted bone integrates to the body structurally and vasculary. Until this time (approximately 4 weeks), the bone graft is unprotected against microbes (exogen and endogen). In orthopedic practice, Staphylococcus aureus and Staphylococcus epidermidis cause periprothetic infections (infection around prothesis). Among these bacteria, polyresistant germs are frequent. The materials, that are used in orthopedic surgery, show different characteristics in the point of view of bacterial adherence.

The appropriate antibiotic has excellent antimicrobial activity, long biological half-life, good tissue penetration. Moreover, it should be nontoxic and relatively cheap. The oral or intravenous administration of antibiotics may cause few side effects. Because of this reason, there is a great demand to develop new carrier systems which may release the antibiotic drug locally. Local antibiotic releasing systems are divided into two groups: biodegradable and nonbiodegradable systems. The main representative of non biodegradable systems is polymethylmethacrylate impregnated with different antimicrobial agents. The group of biodegradable systems contains collagen-gentamicine sponge, hydroxyapatite, polylactide/polyglycolide implants, polylactide polymers, polyurethans. These systems do not need to be removed compared to non biodegradable systems, which reduce the surgical risk of reinfection [3-6].

The aim of our studies was to formulate special drug carrier systems that show extremely prolonged release profile of the applied antibiotic. These formulations should allow the impregnation or coating of the bone grafts (used in revision surgery) and of other biocompatible materials. The controlled release of antibiotics from bone can significantly reduce the prevalence of septic complication of revision operation. Mixing the antibiotic with bone cement, and coating the surface of the bone require a special technology because the release of the active ingredient has to be strictly controlled.

MATERIALS

Vancomycin hydrochloride was purchased from Eli Lilly Hungaria Ltd. (Budapest, Hungary). Carbopols (Carbopol 934 P, 971 P, 980, 974 PNF) were from Noveon Ltd. (Cleveland, USA). Hydrophobic matrix (based on White wax) and Hydroxymetilcellulose were obtained from Hungaropharma Co. (Hungary).

Ingredients of the analytical mobile phase (methanol, ammonium acetate and water Chromosolv for HPLC) were from Sigma Aldrich (Budapest, Hungary).

METHODS

• Preparation of samples

In case of gel systems, Carbopols were poured with distilled water (dissolving Vancomycin hydrochloride), and were kept under 10 ºC for 24 hours. It was neutralized the next day with 1.2 % Sodium hydroxyde solution, therefore gel forming agent arose (50 mg/gel g).

• In vitro test method

For the characterisation of the prepared samples, in vitro releasing tests have been done. In case of gels, dissolution tests were done for 8 hours in phosphate buffer. In case of hydrophobic matrix, the dissolution tests were done for 4 weeks, in physiological salt media. The media has been replaced by fresh media every day.

• Analytical determination

For the determination of the released active ingredient (Vancomycin hydrochloride) from gels in case of phosphate buffer, spectrophotometric method was used (λ=230 nm, U-3501 Spectrophotometer Hitachi Ltd., Tokyo, Japan).

For the determination of the released active ingredient, in case of wax mixtures, isocratic HPLC method was used (Agilent LC MSD 1100 with HP Chemstation software Rev. A10.02, Agilent, Waldbronn, Germany) [7-9].

RESULTS

The experimental dissolution profiles of the samples show, that drug release is influenced by the composition and preparation of drug carrier systems. According to these results, our carrier systems seem to be suitable for the orthopedic use.

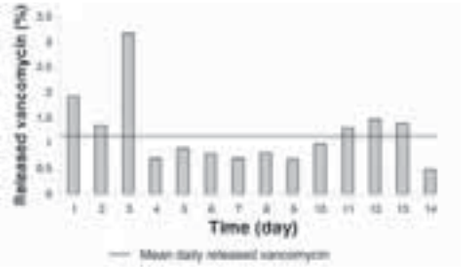

Figure 1: Daily released vancomycin from the prepared hydrophobic matrices

CONCLUSION

The application of polymer gel forming agents influences significantly the release of vancomycin. These materials seem to be advantageous in case of developing modified released drug delivery systems with possible bioadhesivity. Hydrophobic matrices give chance for further development towards the release of antibiotics for few weeks in an effective concentration.

A new analytical method for the determination of vancomycin released from the developed delivery systems has been proved. This method is simple, rapid and able to monitor the controlled release of antibiotics.

ACKNOWLEDGEMENT

The authors thank the Hungarian Scientific Research Fund for the financial support (Grant No.: T049480).

References

1. M. A. Buttaro, A. M. Gonzales Della Valle, I. Pineiro, E. Mocetti, A. A. Morandi, F. Piccaluga, Acta Orthopedica Scandinavica (2003) 74 (5): 505-513
2. C. Chen, J. Ko, C. Pan, Archives of Orthopaedic and Trauma Surgery (2005) 125: 369-375
3. K. Kanellakopoulou, E. J. Giamarellos-Bourboulis, Drugs (2000) 59 (6): 1223-1232
4. J. H. Calhoun, J. T. Mader, Clinical Orthopedics and Related Research (1997) 341: 206-214
5. S. Gitelis, G. T. Brebach, Journal of Orthopedic Surgery (2002) 10: 53-60
6. M. Stigter, j. Bezemer, k. de Groot, R. Layrolle, Journal of Controlled Release (2004) 99: 127-137
7. J. Luksa, A. Marusic, Journal of Chromatography B, (1995) 667: 277-281
8. P. Favetta, J. Guitton, N. Bleyzac, D. Dufresne, J. Bureau, Journal of Chromatography B, (2001) 751: 377-382
9. J. Diana, D. Visky, J. Hoogmartens, A. Van Schepdael, E. Adams, Rapid Communication in Mass Spectrometry (2006) 20: 685-693

PO128

Effect of calcium content on bioactivity and erodibility of Sol-Gel Silica as a matrix for local and sustained release of bioactive compounds

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INTRODUCTION

Sol-gel silica is an inorganic polymer potentially useful as a matrix for the sustained release of drugs. Its synthetic process occurs in mild conditions, it is easy to perform, and the polymer product is versatile in composition and internal network conformation. This allows almost infinite possibilities for modulating the release behavior of embedded compounds. These basic properties come together with the material s unique bioerodibility and low in vivo toxicity, potentially allowing also its use in parenterally administered forms . Current “non-sol-gel” silica-gel products (eg. Aerosil) are used as pharmaceutical excipients but their use is limited to the oral route, their role most commonly being limited to glidants or viscosity modulators. Other silica based materials used in biomedicine are ceramics and fused glass products. The latter have being extensively investigated as bone substitute implants, thanks to their mechanical resistance and bioactivity. These materials are accepted for parenteral administration but, their high temperature of preparation does not permit to embed in them bioactive compounds, a property that, if feasible, would further increase their usefulness.

In a recent work, we investigated a new form of sol-gel silica based polymers to be used for the sustained release of antitumor Pt(II) bisphosphonate complexes to be locally administered upon bone tumor surgical excavation. The composition of the inorganic matrix was planned as to act both as a Pt (II) complex release modulator and as a promoter for bioactivity to favor *in situ* bone tissue reconstruction. The novel hybrid material was capable of controlling the release of the Pt(II) antitumor activity while its bioactivity remained be investigated. In that work the amount of calcium introduced in the composite material was chosen on the basis of what observed with fused bioactive glass and heat cured sol-gel silicas where bioactivity occurs only at or above a Ca:Si molar ratio of 0,33. When this Ca:Si ratio is used in non-heat cured sol-gel silica, the polymer becomes highly porous so that its total drug loading capability is not very high.

In this work we tested the bioactivity and erosion properties of several room temperature processed silica materials differing for their calcium content, from 0 to 0,33 Ca:Si molar ratio. Objective of the study was threefold: a) to verify if the same high calcium as in heat cured materials is necessary for bioactivity of non heat processed gels; b) to evaluate if the amount of calcium in such materials influences silica erosion; c) to test if a reduced calcium content reduces also the calcium release with potential benefits for the sustained release of Ca-binding bioactive compounds.

EXPERIMENTAL

Four sol-gel silica formulations were synthesized at room temperature according to published procedures . The four gels differed for the amount of calcium embedded into them (table 1). After polymerization,

gels were aged in closed vials for three days at room temperature. They were later dried at 50°C until constant weight (about 24h), ground in a mortar and sieved to obtain granule populations with homogeneous distribution of size.

Table 1

Formulation name	Si:Ca molar ratio (mole:mole)
0%	1:0
33%	1 : 0.33
17%	1:0.17
8%	1:0.08

Bioactivity was tested *in vitro* upon immersing the xerogels (size 300-63 μm) at 37°C for 7 days in simulating body fluid (1,5 mg xerogel/ml SBF, corresponding to about xerogel external area of 50 mm^2/ml) [6]. After SBF treatment, the xerogels were rinsed and dried. The presence of HA on their surface was detected by FTIR and XRD.

Silica erodibility and calcium release properties was tested at 37°C, by immersing the xerogels in TBS buffer (10 mMtris/150 mM NaCl, pH 7.4) at silica/buffer ratio of 2mg/1ml buffer. Silicic acid concentration in the release medium was measured by the blue silico-molibdenum test . Calcium concentration was determined by ICP.

RESULTS AND DISCUSSION

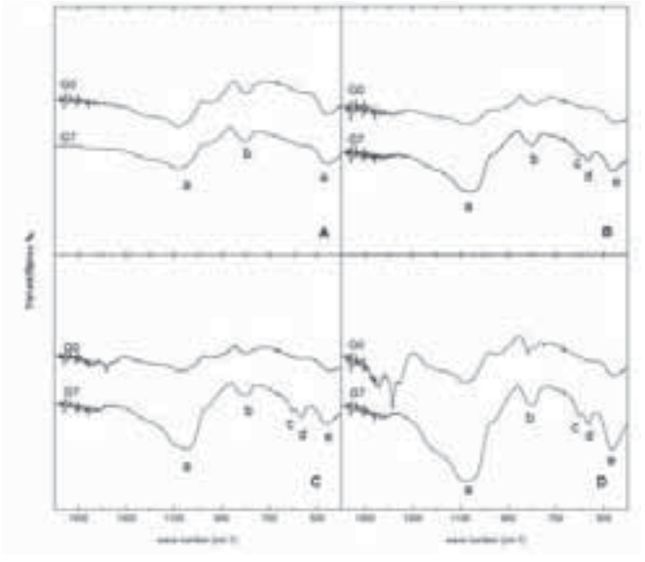

Figure 1: FTIR analyses of calcium free (A) and calcium loaded xerogels 33% (B), 17% (C), 8% (D) before and after SBF treatment. Typical HA signal is the splitted peak (c and d) at around 680 cm-1).

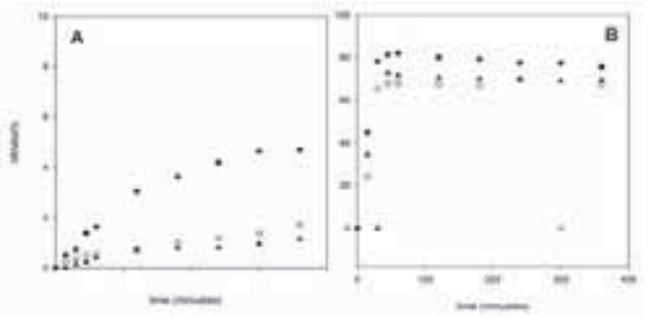

Figure 2: Silica erosion (A) and calcium release (B) profiles of xerogels loaded with different amounts of calcium. Open circle: 8%, triangle 17%, black circle 33%

Figure 1 shows the FTIR analyses of all xerogels before and after SBF treatment. The typical fingerprints of calcium phosphate hydroxyapatite (HA) are visible in all calcium containing samples SBF treated, independently of their initial calcium content. When calcium was not present, HA formation di not occur. These data were also confirmed by XRD analysis which did not indicate significant differences in the degree of crystallinity among the three calcium containing samples. Figure 2 shows the SiO_2 erosion (A) of all samples together with their calcium release (B) profile. It appears that matrix erodibility of the lower calcium content xerogels is slower than in the high calcium ones. On the contrary calcium release occurs fast in all samples, the speed of release being less affected by calcium load.

CONCLUSIONS

All xerogels tested were shown to be bioactive. Therefore heat curing is not necessary for bioactivity of calcium containing silica xerogels. As opposed to high temperature processed silica based material the calcium concentration requirements for effective bioactivity induction are less stringent. According to these results 8% molar concentration (as referred to Si moles), is enough to promote bioactivity.

In addition the amount of calcium present in the formulation affects the material erodibility and when less calcium is present, silica erosion occurs with a reduced rate. Further experiments will be needed to assess the drug releasing properties of these low calcium content xerogels.

References

1. Conconi, M.T., et al., J Biomed Mater Res A, 2008.
2. Teoli, D., et al., Wet sol-gel derived silica for controlled release of proteins. J Control Release, 2006. 116(3): p. 295-303.
3. Hench, L.L.,. J Mater Sci Mater Med, 2006. 17(11): p. 967-78.
4. Martinez, A., et al. Chemistry of Materials, 2000. 12(10): p. 3080-3088.
5. Margiotta, N., et al., Dalton Trans, 2007(29): p. 3131-9.
6. Kokubo, T. and H. Takadama, Biomaterials, 2006. 27(15): p. 2907-15.
7. Koroleff, F., in Methods of Seawater Analysis,, K.K. Grasshoff K, and Ehrhardt M,, Editor. 1983, Wiley-VCH: Weinheim. p. 174-183.

PO129

Evaluation of the floating behaviour and drug release kinetics of various matrix tablets intended for gastroretentive application

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INTRODUCTION

Since the discovery of Helicobacter pylori by Warren and Marshall about two decades ago, more and more attempts have been made to design so called gastroretentive drug delivery systems (GRDDSs). These dosage forms are capable of remaining in the stomach for an extended period of time, which makes them appropriate carriers for drugs used in the local therapy of this organ. For this reason they are promising candidates in the treatment of ulcerative diseases or other local disorders of the upper part of the gastrointestinal tract. They can also be applied for the delivery of drugs having a narrow absorption window located in the stomach or, more probably in the proximal part of the small intestine. Moreover, GRDDSs can be advantageous for molecules showing poor stability or causing adverse effects in the colon (e.g. broad-spectrum antibiotics) [1]. Several pharmaceutical technological solutions have been proposed in the literature to overcome the hurdles of the physiological process of gastric emptying. These involve bioadhesive formulations, floating and high density dosage forms, expandable systems, etc. Probably the most deeply studied of these are floating delivery devices, which remain buoyant upon the gastric contents and thus are kept from passing through the pylorus. The functioning of these systems is either based on gas generation or the addition of low density excipients – leading to a final density less than that of the gastric contents. In both cases, a crucial role can be attributed to polymers applied as matrix-forming agents in such formulations. On the one hand they prolong and govern drug release by swelling and/or erosion, and on the other hand they contribute to the appropriate physical properties (low density) of the dosage form [2]. A large variety of polymers can be applied for the above purposes, such as alginates, cellulose derivatives, polyacrylates, polyethylene oxides, polysaccharides, polycarbonates, polystyrenes, etc. The aim of the present study was to evaluate different types of matrix-forming polymers from the aspect of floating behaviour and drug release kinetics.

MATERIALS AND METHODS

• Materials

The following materials have been used for tablet preparation: metronidazole (Unichem Laboratories Ltd., Maharashtra, India) as a model active, polypropylene foam powder (PP; Accurel MP 1000®, Membrana, Obernburg, Germany) as a low density excipient, calcium-carbonate and sodium-hydrogencarbonate (CC and SHC; Hungaropharma, Budapest, Hungary) as gas-generating agents, hydroxypropylmethycellulose (HPMC4 and HPMC15; Methocel K4M and K15M CR Premium®; Colorcon, Dartford Kent, United Kingdom;) and poly(ethyleneoxide) (PEO303 and PEON-12K; Polyox WSR 303® and Polyox WSR N-12K®; The Dow Chemical Company, Midland, USA) as matrix-forming polymers.

• Tablet preparation

Tablets were prepared by direct compression using a Diaf (Denmark) single punch press. Besides metronidazole, formulations $F_{1/1}$-$F_{1/9}$ contained HPMC4, PEO303 and PEON12K in different ratios, and equal amounts of CC and SHC (gas-generating systems). Formulations $F_{2/1}$-$F_{2/9}$ were composed of the active ingredient, HPMC4, HPMC15 and PP foam powder (low-density systems).

• Evaluation of the tablets

The floating behaviour of the tablets was monitored visually, and formulations that proved to have appropriate hydrodynamic properties (i.e. became afloat in 30 minutes) were also tested from the aspect of drug release properties. For this purpose an Erweka DT6RE type dissolution tester was applied at 37±1°C and 50 rpm, using rotating baskets. The dissolution medium was 500 ml of 0.1 N HCl with 2g/l of sodium chloride. The active content of the samples was determined with a Jasco V-550 UV/VIS spectrophotometer at 280 nm. Different models (zero-order, first-order, Higuchi's and Ritger & Peppas') were fitted to the dissolution curves, and correlation with the measured data was calculated.

RESULTS AND DISCUSSION

Among gas-generating formulations, only the ones lacking HPMC4 were capable of floating within 30 minutes. As all tablets were prepared using the same compression force, this might be attributed to higher compressibility and thus higher density of formulations containing the fibrous cellulose derivative. Figure 1 shows the drug release profiles of the remaining three compositions ($F_{1/1}$-$F_{1/3}$), which comprised only PEOs as matrix-forming agents. Drug release rate was the highest for $F_{1/1}$ containing only the lower molecular weight form (PEON-12K), and it decreased as the ratio of the higher molecular weight form (PEO303) increased. Although the release profiles seem to be close to linear kinetics, the best correlation (0.9998 in all three cases) was found with the model of Ritger and Peppas. Floating behaviour proved to be independent of the molecular weight of the matrix forming polymer.

As HPMC4 did not present appropriate density in gas-generating formulations, the addition of a low density excipient (PP foam powder) was attempted instead of CC and SHC. A higher molecular weight form of HPMC (HPMC15) was also tested in this system. Similarly to PEOs, no correlation between floating behaviour of the tablets and molecular

weight of the polymer was found. Formulations $F_{2/1}$-$F_{2/3}$ did not contain the low density excipient, and thus did not become buoyant. Compositions $F_{2/7}$-$F_{2/9}$ contained 35% of the foam powder, which caused inhomogeneity of the tablets because of the great difference in the density of the components.

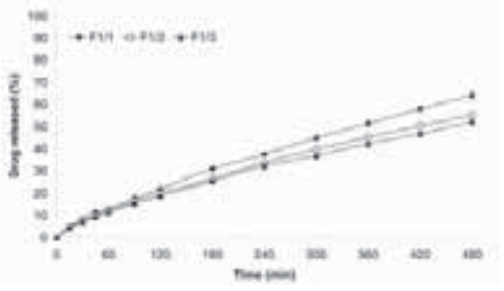

Figure 1: Drug release profiles of gas-generating systems

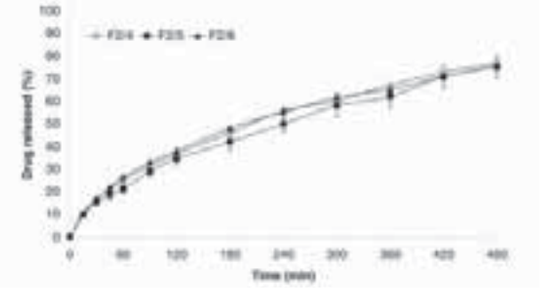

Figure 2: Drug release profiles of low density systems ($F_{2/4}$ – HPMC15, $F_{2/5}$ – HPMC4 and HPMC 15, $F_{2/6}$ – HPMC4)

For this reason, only $F_{2/4}$-$F_{2/6}$ were studied from the aspect of dissolution properties (Figure 2). It can be seen that drug release profiles of HPMC matrix tablets are closer to first-order kinetics than that of PEO matrices. The correlation in two of three cases however was the best with the release model of Ritger and Peppas. It could also be stated, that there was no significant change in the drug release rate of the higher and lower molecular weight HPMCs.

CONCLUSION

The evaluation of the drug release profiles of matrix tablets meant for gastroretentive application has revealed that PEO type matrix-formers show better hydrodynamic properties than HPMCs. It can also be stated that the drug release profile of PEOs is closer to zero-order kinetics, while that of HPMCs is rather first-order. No correlation was found between the floating behaviour and the molecular weight of the polymers.

References

1. Singh BM, Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J Con Rel, 63: 235-259 (2000).
2. Gerogiannis VS, Rekkas DM, Dallas PP, Choulis NH. Floating and swelling characteristics of various excipients used in controlled release technology. Drug Dev Ind Pharm, 19: 1061-1081 (1993).

PO130

Evaluation of guar gum and xanthan gum as compression coat for colon specific drug delivery

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INTRODUCTION

Drug targeting to colon is highly desirable in a variety of colonic disorders such as inflammatory bowel diseases, infectious diseases, Crohn's Disease and colon cancer. Various approaches have been used for targeting the drugs to the colon including, formation of a prodrugs, time-dependent delivery systems, coating with pH-sensitive polymers, pressure-dependent systems, and systems formulated with biodegradable polymers. However, biodegradable systems formulated with natural polysaccharides are increasingly being developed. Natural biodegradable polysaccharides such as guar gum, xanthan gum, chitosan and pectin remain undigested in the stomach and the small intestine and are degraded by the anaerobic microflora of the colon (1-4).

The aim of this study is to evaluate colon specific drug delivery using in vitro methods. Tablets were prepared using pectin, guar gum and xanthan gum as biodegradable polysaccharides. Ornidazole, was used as a model drug.

MATERIALS AND METHODS

Materials

Ornidazole was a gift from Abdi İbrahim İlaç San. ve Tic. A.Ş. (Turkey). Guar gum and xanthan gum were obtained from Droguan A. L. Wolf GmbH (Germany) and Jungbunzlauer (Avustria). Pectin was donated by Wyeth İlaçlari A. Ş. (Turkey). Pectinex 3XL was obtained from Novo Nordisk (Switzerland). All other solvents and reagents were of analytical grade.

Preparation of Tablets

Ornidazole was dry mixed with PVP and granulated with water. The granules prepared were sieved and dried overnight at room temperature. Core tablets were compressed with 10 mm flat faced punches using a hydraulic hand press tablet machine (Perkin-Elmer) with a pressure to 5.0 tons for 10 seconds.

Rapidly disintegrating core tablets containing 200 mg ornidazole were compression coated with 400 mg of a mixture of pectin and guar gum (GG) or pectin and xanthan gum (XG) in varying proportions.

To prepare the coated tablets, half of the coating material was placed in the 13 mm die cavity of the flat faced punches. The core tablet was positioned in the centre of the die cavity and filled with the other half of the coating material. The coating material was compressed around the core at an applied force of 5.0 tons for 10 seconds. Composition of coat formulation is given in Table 1 (5).

Table 1: Composition of coat formulation

Formulation Code	Pectin (%)	Guar Gum (%)	Xanthan Gum (%)
F1	80	20	-
F2	60	40	-
F3	80	-	20
F4	60	-	40

Tablet quality control tests

Tablet quality control tests were performed on the core tablets and compression coated tablets according to USP 24 (6).

In vitro dissolution studies

In vitro dissolution studies were performed by paddle method according to USP 24 with 50 rpm paddle speed and 900 mL at 37°C. The drug release from tablets were tested for 2 hours in pH 1.2 hydrochloric acid buffer as the average gastric emptying time is approximately 2 hours. Then the dissolution medium was replaced with pH 6.8 phosphate buffer, and the study was performed for 3 hours as the average small intestinal transit time is about 3 hours.

At the end of 5 hours 9 mL pectinolytic enzyme was added to dissolution medium and test continued for 24 hours. The dissolution studies were carried out with and without enzyme to determine the effect of pectinolytic enzyme. The amount of ornidazole released from tablets at different time intervals was estimated by spectrophotometrically (n=3).

Swelling Studies

Swelling studies of tablets were performed under conditions identical to dissolution testing. At predetermined time intervals, tablets were carefully removed from the dissolution vessels, excess surface water was lightly removed and wet weights of tablets were determined gravimetrically.

RESULTS AND DISCUSSION

Tablet quality control tests

From tablet quality control studies, it was found that all tablets were high quality and complied with pharmaceutical standards. Table 2 shows the results of tablet quality control studies.

Table 2. Physical properties of core and compression coated tablets

Codes	D/T	W	C. S.	F	C. U.
Core Tablet	3.576 ±0.036	0.202 ±0.001	4.585 ±0.234	0.491	98.452 ±0.563
F1	3.589 ±0.036	0.603 ±0.001	6.254 ±0.045	0.447	93.345 ±0.342
F2	3.603 0.065	0.603 ±0.001	5.154 ±0.067	0.100	94.234 ±0.564
F3	3.654 ±0.030	0.603 ±0.001	4.843 ±0.054	0.202	93.872 ±0.845
F4	3.631 ±0.066	0.604 ±0.001	6.277 ±0.070	0.222	97.726 ±0.384

D/T: Diameter/Thickness, W: Weight Variation (g),
C.S.: Crushing Strength (kg/monsanto), F: Friability (%),
C. U.: Content Uniformity (%)

In vitro release studies

From the in vitro drug release studies, it was found that ornidazole was not released from tablets in simulated gastric and intestinal fluids. The use of pectinolytic enzymes showed that the pectin/guar gum and pectin/xanthan gum mixtures were susceptible to enzymatic breakdown. The release of ornidazole from compression coated tablets is shown in Figure 1 and Figure 2.

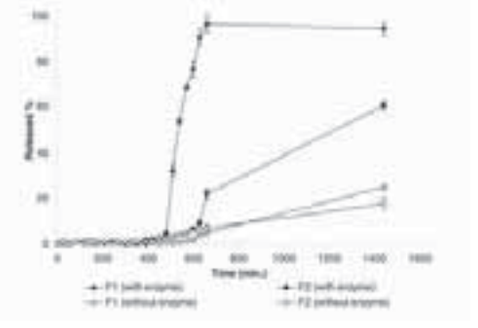

Figure 1: The release of ornidazole from tablets coated with pectin and guar gum

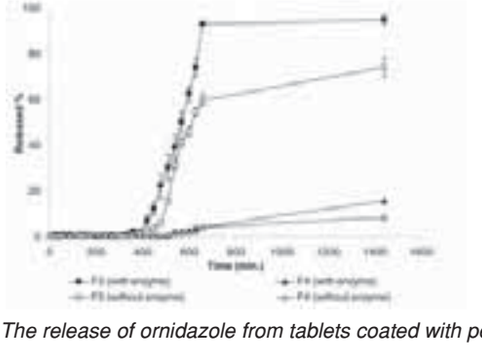

Figure 2: The release of ornidazole from tablets coated with pectin and xanthan gum

Swelling Studies

Swelling studies showed that compression coat of the tablets hydrated and formed viscous gel layer around core tablets. Weight change of formulations is shown in Figure 3 and 4.

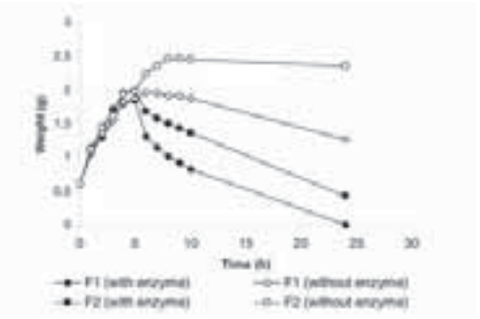

Figure 3: Weight change of tablets coated with pectin and guar gum

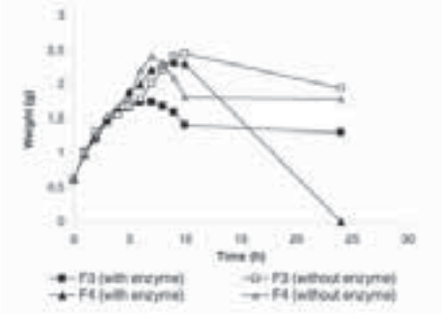

Figure 4: Weight change of tablets coated with pectin and xanthan gum

CONCLUSION

In vitro release studies have shown that guar gum and xanthan gum in the compression coat protect the drug being released under conditions mimicking mouth to colon transit. Studies in pH 6.8 phosphate buffered saline containing 1% pectinolytic enzymes have demonstrated the susceptibility guar gum and xanthan gum to the colonic bacterial enzyme degradation.

The study clearly established that guar gum and xanthan gum in the compression coat, are potential carriers for drug targeting to colon.

References

1. Sinha V.R., Mittal B.R., Bhutani K.K., Kumria R., Colonic drug delivery of 5-fluorouracil: an in vitro evaluation. Int. J. Pharm. 269: 101–108 (2004).
2. Krishnaiah Y.S.R., Satyanarayana S., Rama Prasad Y.V., Narasimha Rao S. Evaluation of guar gum as a compression coat for drug targeting to colon. Int. J. Pharm. 171: 137–146 (1998).
3. Gliko-Kabir I., Yagen B., Baluom M., Rubinstein A. Phosphated crosslinked guar for colon-specific drug delivery II. In vitro and in vivo evaluation in the rat. J. Control. Rel. 63: 129–134 (2000).
4. Sinha V.R., Kumria R. Binders for colon specific drug delivery: an in vitro evaluation. Int. J. Pharm. 249: 23-31 (2002).
5. Turkoglu M., Ugurlu T. In vitro evaluation of pectin-HPMC compression coated 5-aminosalicylic acid tablets for colonic delivery. Eur. J. Pharm. Biopharm. 53: 65-73 (2002).
6. The United States Pharmacopeia, The National Formulary, USP XXIV, NF XIX, Supplement Two, Convention Inc., Rockville, (2000).

PO131

Validation of the laboratory – scale techological process preparation of a colonic release pharmaceutical system with pH and time-control

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INTRODUCTION

Although oral delivery has become a widely accepted route of administration of therapeutic drugs, the gastrointestinal tract presents several formidable barriers to drug delivery. Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon (Chron's diseases, ulcerative colitis, colorectal cancer and amebiasis) but also for its potential for the delivery of proteins and therapeutic peptides [1,2].

The development and the design of colon-specific drug formulations represents a technological challenge as these dosage forms must pass through the upper gastrointestinal (GI) tract in intact form before delivering the drug to the colon. To achieve successful colonic delivery, a drug needs to be protected from absorption and /or the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs [1,2]. Validation of a manufacturing process is to ensure that the manufacturing process does what it purports to do. Pharmaceutical process validation guarantees the reliability and reproducibility of the manufacturing process.

The aim of this experimental works was to validate the technological process of preparation at laboratory scale one colonic delivery pharmaceutical system based on pH and time - control release device. The following steps of technological process were validated: powder blending, tabletting, coating with Eudragit RS and coating with Eudragit L 55. For each step of pharmaceutical process were established the critical process parameters, the responses (with method of evaluation) and acceptance criteria.

EXPERIMENTAL METHODS

Materials. Indomethacin (Terapia SA, România), microcrystalline cellulose PH 102 (JRS Pharma, Germany), lactose monohydrate (Borculo, Netherlands), polyvinylpyrrolidone K_{25} (BASF, Germany), talcum (S&D Chemicals, UK), magnesium stearate (Union Derivan, Spain), Eudragit RS 30D, Eudragit L 5530D (Rohm Pharma, Germany), triethyl citrate (Merck, Germany), hydroxypropyl methylcellulose (Methocel E 15LV) (Colorcon, UK), titan dioxide (S&D Chemicals, UK), Simeticone (Colorcon, UK).

Apparatus. Tablet press EK-0 (Korsch, Germany), planetary mixer PRS (Erweka, Germany), fluid bed system Strea 1 (Aeromatic, Switzerland), UltraTurax (Janke and Kunkel, Germany), dissolution apparatus PT-DT7 apparatus no. 1 (basket) (PharmaTest, Germany), spectrophotometer UV-Vis Jasco V530 (Jasco, Japan).

Minitablets preparation and characterization. The minitablets were obtained via direct compressing using a Korsch EK 0 eccentric press equipped with die and punches of 5mm. The machine was adjusted to obtain minitablets of 75 mg weight and hardness of minimum 6kg force. The tablets were evaluated for hardness, friability and mass uniformity using methods described in PhEur 5.

The minitablets were coated with polymeric films (Eudragit RS 30D, Eudragit L 55 30D), in a fluidized bed coating system (Strea 1, Aeromatic Filder).

***In vitro* release studies.** Dissolution media: 900 ml 0.1N HCl solution (first 2 h) and phosphate buffer pH 6.8, (next 22h); rotation speed – 50rpm; temperature 37 ± 0.5 °C

RESULTS AND DISCUTIONS

The colonic delivery pharmaceutical system consists in minitablets with indomethacin as model drug and sodium starch glycolate, as swelling agent, coated with a retardation layer that contains: an interior polymeric film insoluble and permeable (Eudragit RS) and an exterior polymeric enteric layer soluble pH – dependent (Eudragit L 55).

The optimum formula that was used in validation program was determinated using a central composite experimental design. The structure of the colonic release pharmaceutical system is presented in figure 1.

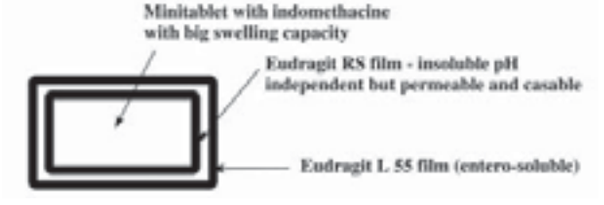

Figure 1: The structure of the colonic delivery pharmaceutical system

The results from tablet preparation steps show that the pharmaceutical process are robust and allow obtaining, in reproducible condition, tablets with very good pharmacotechnical properties.

Table 1: Results obtain at powder blending

No. Crt.	Batch	Samp No.	Powder homogeneity mg	%		CV
	Requirements		-	90-110%		CV < 5
1	MCC001	1	10.23	102.34	Mean	2.711
		2	10.44	104.4	10.1	
		3	9.943	99.43	SD	
		4	9.784	97.84	0.27	
		5	9.885	98.85		
2	MCC002	1	9.555	95.55	Mean	2.94
		2	10.14	101.4	10	
		3	10.35	103.5	SD	
		4	9.973	99.73	0.29	
		5	10.02	100.2		
3	MCC003	1	10.23	102.3	Mean	2.628
		2	10.52	105.2	10.1	
		3	10.03	100.3	SD	
		4	9.984	99.84	0.27	
		5	9.834	98.34		

Table 2: Results obtained at friability and crushing strength determination

No. Crt.	Batch	Friability (%)	Crushing strength (kg)		
	Requirements	Max. 0.2%	minim 6 kg		
1	MCC001	0	9	7.5	Mean
			8	8.5	8.1
			8	8	
			8	8.2	Min
			8	7.5	7.5
2	MCC002	0.098	8	7	Mean
			8	8	7.9
			7.5	8	
			8	8	Min
			8.5	8	7
3	MCC003	0.099	6	8	Mean
			8	6	7
			6	6	
			7.5	8	Min
			7.5	7	6

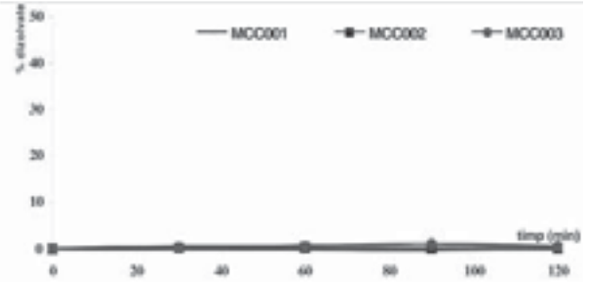

Figure 2: Indomethacin release form colonic delivery pharmaceutical system in 0.1N HCl media

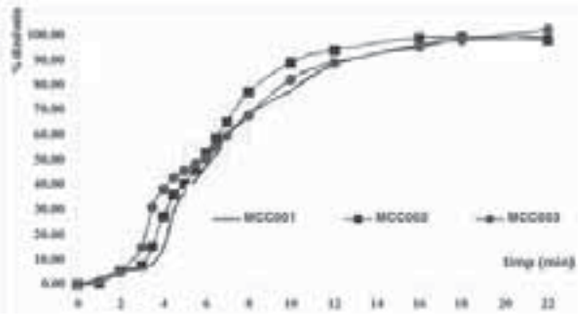

Figure 3: Indomethacin release form colonic delivery pharmaceutical system in phosphate buffer pH 6.8 media

Table 3: Dissolution profile comparison in phosphate buffer pH 6.8 media the validation batches

Validation Batches	f2
MCC001 - MCC002	60,15
MCC001 - MCC003	51,95
MCC002 - MCC003	58,49

The results obtained at dissolution studies on the validation batch are very similar and in accordance with the parameter established during the colonic release system formulation and optimization.

CONCLUSIONS

The results obtained on three validation batches are very similar in all steps of pharmaceutical technological process. More, all the results comply the acceptance criteria established for each critical process parameter in validation protocol.

In conclusion, the result obtained on three laboratory scale batches made in different days show that lab-scale technological process are reliable and reproducible.

References

1. Chourasia M. K., Jain S. K., *Pharmaceutical approaches to colon targeted drug delivery systems*, Pharm. Pharmaceut. Sci. 6(1):33-66, (2003).
2. Leopold C.S., Eikeler D., *Basic Coating Polymer for the Colon-Specific Drug Delivery in Inflammatory Bowel Disease*, Drug Development and Industrial Pharmacy, 26 (12):1239-1246, (2000).

PO132

An *in vitro* investigation into the suitability of bacterially triggered delivery system for colon targeting to deliver Anti Hypertensive agent

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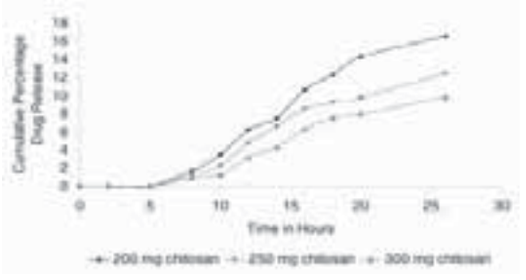
INTRODUCTION

The aim of the drug delivery system targeted to the colon is not only to protect the drug from being released in the physiological environment of stomach and small intestine, but also to release the drug in the colon after enzymatic degradation of colonic bacteria. Several studies were undergone on the basis of the activity of colonic bacteria on polysaccharide based carrier system. Over 400 species of bacteria found, predominantly anaerobes and a small number of fungi. The bacterial count (colony forming unit/mL, CFU/mL) in different regions of the GIT is 0-10^3 CFU/mL in stomach, 0-10^5 CFU/mL in jejunum: 10^3-10^7 CFU/mL in ileum and 10^{11}-10^{12} CFU/mL in colon. Most of them are anaerobes. Eg: Bacteroides, Bificlobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Ruminococcus, Propionibacterium and Clostridium; others are facultative anaerobes eg: E.Coli. Among all of them 20-30% are Bacteroides. The principal sources of nutrition for the colonic microorganisms are carbohydrates arriving in intestinal chyme. When the dosage form reaches the large intestine the bacterias present will take these polysaccharides and release the drug to the colon environment. Where the drugs absorbed by the large intestine wall to systemic circulation. The different polysaccharides that are used under evaluation as carriers for colonic drug delivery includes pectin and its salts chondroitin sulphate, amylase, inulin HP, guar gum. The locust bean gum or Ceratonia is neutral polysaccharides having a molecular weight of 310000 derived from the endosperm of the seed of the ceratonia siliqua linne (Fam: leguminosae). The locust bean contains about 88% D-galacto-D-mannoglycan, 4% of pentan, 6% of protein, 1% of cellulose and 1% of ash. Chitosan is a non-acetylated or partially acetylated chitin derivative. Crustacean shells are the usual raw material for chitin. Chitosan is 2 amino-2-deoxy b-D-glucan. It is tough, bio-degradable and non-toxic. In pharmaceutical field, locust bean gum is used as an excipient for tablets and thickener for tooth paste while the chitosan is used as an excellent direct compression aid and as a vehicle to enhance the dissolution of poorly absorbable drug. In the present study, the locust bean gum and chitosan were applied over the core tablet in the form of compression-coat and evaluated as a carrier for colon specific drug delivery. The *in vitro* drug release studies were carried out in the simulated gastrointestinal fluids in the presence and absence of rat caecal content.

FORMULATION OF PRESS COATED TABLETS

Core tablets of Propranolol HCl were formulated Using 6 mm round, flat and fine punches on a single station tablet machine (Rimex mini press, India) with the thickness of 2 mm. The Compression coating of Propranolol Hcl tablet prepared by placing half the quantity of the coating materials (Chitosan, and Locust Bean Gum) in different amount in the die cavity, the core was carefully positioned in the center of the die cavity, and was filled with other half of the coating material. The coating material was compressed around the core at an applied force of 5000 kg using 12 mm round, flat and plain punches.



Graph No 3: Mean (SD) Cumulative percentage release of Propranolol Hcl on various concentrations of Chitosan (200 mg, 250 mg, 300 mg) without rat ceacal content

Evaluation of Tablets

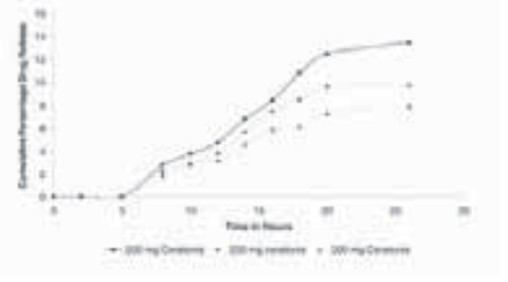
All the tablets prepared were found to contain the medicament within 100 5% of labeled claim. Hardness of the tablets in all the batches was found to be in the range of 6.0-6.5 kg/cm^2 and was satisfactory. The percentage weight loss in the friability test was found to be less than 1% in all the batches

***In vitro* Release Study in simple buffer**

The drug release study was performed with Dissolution rate test apparatus (USPXXIII) (apparatus 1,100 RPM, 37^{0}C), The tablets were tested for drug release for 2 hrs in 0.1N Hcl (900 ml), Then the dissolution medium was replaced with pH 7.4-phosphate buffer (900 ml) and tested for drug release for 3 hrs. Then the dissolution medium was replaced with pH 6.8-phosphate buffer. The drug release studies were carried out for 21 hours and 1ml samples were taken at different time and replaced with 1 ml of pH 6.8 PBS. The result shows the release of the drug from the tablet coated with Locust Bean Gum is comparable with the drug release from tablet coated with chitosan ensures all these formulations were to be found intact retaining their coats and slight swelling of coats due to water sorption were observed percent drug released from the Propranolol Hcl tablet was less in 7.4 pH buffer than the percentage of the drug released in 6.8 pH buffer phosphate.

***In vitro* Release Study with 2% Rat Caecal buffer**

To induce bacterial enzymes postulated to be in the caecum, five Wister rats, weighing 200-300 gm were in tubulated with Teflon tubing for 7 days before the release experiments were initiated. Each day 1ml of 2% w/v of polymer dispersions (i.e., Chitosan / Locust Bean Gum) was administered to the rat's stomach through the Teflon tubing. This process provides the best condition for in vitro evaluation. 30 min before the commencement of the drug release studies, five rates were killed by spinal traction, the abdomen were opened, the caecai were isolated, ligated at both ends, deselected and immediately transferred in to pH 6.8 buffer previously bubbled with CO_2.The caecal bags were opened; their contents were individually weighed pooled and then suspended in PBS to give a final caecal dilution of 2% w/v. As caecum is naturally anaerobic, all these operations were carried out under CO_2. These studies on the Locust Bean Gum as a coating material proved capable of protecting the core tablet containing propranolol HCl during the condition mimicking mouth to colon transit as like chitosan. In particular, the formulation containing Locust Bean Gum 300 mg better dissolution profile, hence a potential carrier for drug targeting to colon.



Graph No 4: Mean (±SD) Cumulative percentage release of Propranolol Hcl on various concentrations of Ceratonia (200 mg, 250 mg, 300 mg) without rat ceacal content

CONCLUSION

The results from the study clearly show that Locust Bean Gum in the form of a compression coat is a potential barrier for drug targeting to the colon like chitosan. The polysaccharide is capable of retarding the release of the core materials until they reach the colon.

Reference:

1. Chellan Vijaya Raghavan, *Chem. Pharm. Bull.* **50**(7) 892—895 (2002).
2. Taniguchi K., Muranishi S., Sezaki H., *Int. J. Pharmaceut.*, **4**, 219 (1980).

PO133

Diffusion of captopril from a matrix type transdermal therapeutic system

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INTRODUCTION

Captopril, an orally effective angiotensin I converting enzyme inhibitor, is used in chronical treatment of hypertension and congestive heart failure as first agent, because of the absence of side effects in the majority of patients. It has a relatively short elimination half life in plasma (2 h) and low oral bioavailability (60-75%) [1]. For these reasons, by applying this drug as a transdermal therapeutic system, dosing intervals will expand so that patient compliance will arise and side effects will be minimised. Transdermal patches are designed to support the passage of drug substances from the surface of the skin, through its various layers and into the systemic circulation. They have been developed with the objective of overcoming the hepato-gastrointestinal first pass metabolism, duplicating the benefits of intravenous drug infusion and achieving systemic rate-controlled drug delivery [2]. In the cases where oral delivery is contraindicated or when the drug is poorly absorbed from the gastrointestinal tract, transdermal route of drug administration may be used. The aim of our study is to prepare captopril containing transdermal therapeutic formulations and evaluate the diffusion rates of captopril from these formulations using various synthetic membranes, animal and human skin.

EXPERIMENTAL METHODS

• Preparation of the Transdermal Therapeutic Systems

Captopril containing transdermal therapeutic systems were prepared according to a previous study [3]. To prepare these formulations, the required amounts of plasticizer (PEG 400) and polymer (Eudragit RL 100 and/or Eudragit RS 100) were dissolved in acetone as casting solvent, then captopril solution in acetone was added and stirred by using magnetic stirrer (RO 5 power IKA Labortechnik). A glass mould of 5 cm diameter was coated with aluminium foil as impermeable backing layer. The solution prepared was poured into this mould and was allowed to dry at room temperature. Acetone was used in the minimum amount enough to solve the polymer and the drug. In formulations containing penetration enhancers, the preparation method was the same, except that the required amount of enhancer was added just before pouring into the mould. The formulations containing polyisobutylene (PIB) adhesive layer were prepared by adding the solution of PIB in 15 ml hexane on to the dry transdermal film prepared and was allowed to dry at room temperature. The composition of the formulations are listed in Table 1.

• Diffusion Studies

Diffusion experiments were conducted using Franz diffusion cells that have a receptor volume of 31 ml and a diffusional area of about 3.14 cm^2. The receptor chambers have side arms through which samples could be taken. Deaerated distilled water was used as the receptor phase. Various synthetic membranes with different properties (Table 2) were used as the diffusion surface. The receptor compartment of the cells was maintained at 32ºC. Teflon coated magnets were used to agitate the receptor compartments to provide uniform mixing . Sink conditions were maintained throughout the experiment. At predetermined intervals, samples were taken from the receptor part and replaced with an equal volume of receptor phase. All samples were assayed using UV spectrophotometer.

Table 1: The composition of matrix type transdermal therapeutic systems containing captopril.

	FM1	FM2	FM3	FM4	FM5	FM6	FM7	FM8
Eudragit RL 100 (g)	2	1+ 1	1,5	1+ 0,65	1,3	1,3	1+ 1	2
Eudragit RS 100 (g)	_	_	0,5	0+ 0,35	0,7	0,7	_	_
PEG 400 (g)	0,4	0,2+ 0,2	0,4	0,2+ 0,2	0,4	0,4	0,2+ 0,2	0,4
Captopril (g)	0,4	0,2+ 0,1	0,4	0,2+ 0,1	0,4	0,4	0,2+ 0,1	0,4
Acetone (ml)	7+ 5	7+ 5	7+ 5	7+ 5	7+ 5	7+ 5	7+ 5	7+ 5
PIB (g)	0,5	0,5	0,5	0,5	0,5	0,2	0,2	0,1
Hexane	15	15	15	15	15	15	15	15

Table 2: Characteristics of the synthetic membranes used.

Type of membrane	Pore size (μm)	Thickness (μm)	Nature
GVHP	0,22	125	hydrophobic
FHLP	0,45	175	hydrophobic
HAWP	0,45	180	hydrophilic
VCWP	0,1	105	hydrophilic

RESULTS AND DISCUSSION

Diffusion profiles of captopril from the transdermal therapeutic system were shown in Figure 1 and 2 respectively.

As shown in Figure 1, the diffusion rate of captopril from the transdermal therapeutic system when using HAWP membrane was lower when compared with VCWP membrane. This may be attributed to the thickness of the membranes (Table 2). Diffusion rates of captopril when using hydrophobic membranes were slower than the rates found when using hydophilic membranes (Figure 1, 2). The reduced amount of PIB in the formulations (FM 6, FM7, FM 8) affected the diffusion profiles.

According to this effect, significant increase on the diffusion profile of captopril from these formulations was seen as shown in Figure 2.

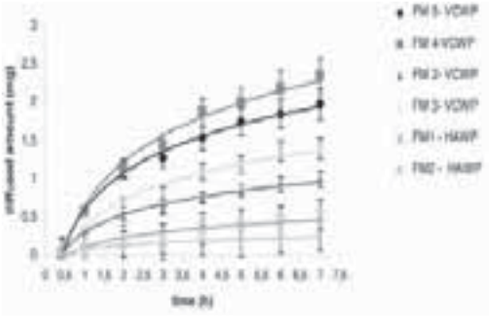

Figure 1: The effects of hydrophilic membranes on the diffusion of captopril from matrix type transdermal systems

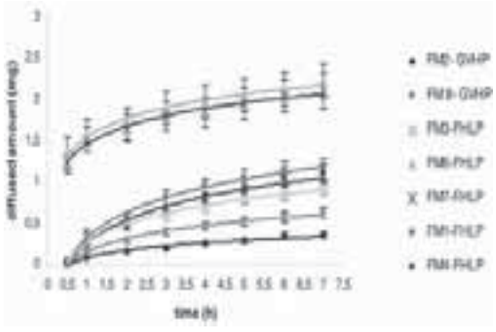

Figure 2: The effects of hydrophobic membranes on the diffusion of captopril from matrix type transdermal systems

CONCLUSION

Type of synthetic membrane used and the composition of the formulations affected the diffusion profiles of captopril from the transdermal therapeutic systems. For further studies, various animal skins and human skin will be used as diffusion surface in order to compare the results obtained with the synthetic membranes.

ACKNOWLEDGEMENTS

This study was supported by Marmara University Research Fund (Project no: SAG-YLS-120707-0127)

References

1. Martindale The Extra Pharmacopoeia, 29th edition; The Pharmaceutical Press, London, 1989.
2. Wokovich, A.M., Prodduturi, S., Doub, W.H., Hussain, A.S., Buhse, L.F., Transdermal drug delivery system (TDDS) adhesion as a critical safety, efficacy and quality attribute, Eur. J. Pharm. Biopharm., 64, 1-8, 2006.
3. Natan, Ş.: Preparation and in Vitro Evaluation of Captopril Containing Matrix Type of Transdermal Therapeutic System, Master Thesis, Marmara University, Istanbul, Turkey, 2002

PO134

Screening for the preparation conditions for an O/W/O multiple emulsions with Eusolex 6007 using Placket-Burman experimental design

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INTRODUCTION

Multiple emulsions are complex dispersion systems, known also as "emulsions of emulsions". These emulsions systems have significant potential in pharmacy and cosmetics. In cosmetics several attractive properties are usually reported: a high capacity of entrapment compared to other systems, the protections of fragile substances, the possibility of coexistence of two immiscible agents in the same product and the possibility of providing prolongation of drug release by including drugs in the innermost phase. The homogeneity of these systems and an accurate control of internal structure are critical for the versatility of such emulsions, because these attributes allow precise manipulation of the loading levels and the release and transport kinetics of the encapsulated substances [1-4]. There are several factors affecting the stability of multiple emulsions, including the method of preparation, the concentration and type of emulsifiers, the additives which may improve the stability of these systems.

The objective of this study was to formulate and characterize an $O_1/W/O_2$ multiple emulsion, of second order and three components, containing a sunscreen agent (Ethylhexyl dimethyl PABA, Eusolex 6007, a stable UVB filter). A screening procedure was used in order to find the best preparation conditions for the multiple emulsions.

EXPERIMENTAL METHODS

Material and composition

Eusolex 6300 was selected as active ingredient of the multiple emulsions. The lipophilic surfactant, sorbitan monostearate (Span 60), the hydrophilic emulsifier, polyoxyethylene (20) sorbitan monooleate (Tween 80), the cetyl alcohol, stearyl alcohol, polyethylene glycol 6000 (PEG 6000) and Eusolex 6300 were supplied by Merck (Germany). Paraffin, paraffin oil and aluminium stearate were purchased from Romaqua Holdings (Romania). All other chemicals used for analysis were analytical grade.

Emulsion preparation

A two-step procedure was used to prepare the multiple emulsions. UltraTurax (Janke and Kunkel, Germany) was used for primary emulsification step and DLS Stirrer (Velp Scientifica, Germany) for the second emulsification step.

In the first step, we prepared the O/W primary emulsion. We heated separately the aqueous phase and the oil phase containing the Eusolex 6007. The oil phase was added in the aqueous phase under high speed agitation (1000-10000 rpm) and at high temperature at $50^0C \pm 2^0C$, for different time intervals. In the second step the O/W primary emulsion was added to the second oil phase heated at 70^0C containing the lipophilic surfactant and then agitated the multiple emulsion at lower speed (500 – 2000 rpm) and 50^0C, for different time intervals.

Emulsion characterization

Microscopic analysis. The microscopic analysis provides information about the multiple characters of the emulsion, the size and the homogeneity of the drops. This was performed with an optical microscope (Optika, Italy) with a videocamera (Optikam 3 digital camera). The droplet size was measured using the microscope software.

Rheological measurements were performed on a rotational rheometer (Brookfield DV-III Ultra) at 22 ± 0.2^0C.

Software. Experimental design, coefficient calculation, statistic parameters calculation and evaluation of quality of fit were performed with Modde 6 software, (Umetrics, Umea, Sweden).

Experimental Design. To perform this study, a Plackett-Burman fractional experimental design with nine variables and two levels was used. The studied variable were: the homogenizer type (X_1), the stirring time for the first (X_2) and second emulsification step (X_4), the stirring rate for the first (X_3) and for the second emulsification step (X_5), the ratio of hydrophilic (X_6) and lipophilic (X_7) surfactant, the percent of lipophilic phase in the primary emulsion (X_8) and the percent of aluminium stearate (X_9) in the multiple emulsion.

The responses variables were: the particle size (Y_1) and the polydispersion index (Y_2) for the internal oil drop drop, the particle size (Y_3) and the polydispersion index (Y_4) for the water drop and the viscosity of the multiple emulsion (Y_5). The matrix of experimental design is presented in table I.

Table 1: Matrix of experimental design

Exp Name	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
N1	B	20	10000	5	500	1	5	15	2
N2	B	60	1000	30	500	1	1	15	2
N3	A	60	10000	5	2000	1	1	5	2
N4	B	20	10000	30	500	5	1	5	0
N5	B	60	1000	30	2000	1	5	5	0
N6	B	60	10000	5	2000	5	1	15	0
N7	A	60	10000	30	500	5	5	5	2
N8	A	20	10000	30	2000	1	5	15	0
N9	A	20	1000	30	2000	5	1	15	2
N10	B	20	1000	5	2000	5	5	5	2
N11	A	60	1000	5	500	5	5	15	0
N12	A	20	1000	5	500	1	1	5	0
N13	A	40	5500	17.5	1250	3	3	10	1
N14	A	40	5500	17.5	1250	3	3	10	1
N15	A	40	5500	17.5	1250	3	3	10	1

X_1 - type of homogenisator; X_2 - time of stirring (primary emulsion); X_3 - stirring rate (primary emulsion); X_4 - time of stirring (multiple emulsion); X_5 - stirring rate (multiple emulsion); X_6 - ratio of hidrophylic surfactant; X_7 - ratio of lipophilic surfactant; X_8 - lipophilic phase ratio, X_9 – aluminium stearate ratio.

RESULTS AND DISCUTIONS

Experimental design analysis. Goodness of fit.

The matrix of the results is shown in Table II.

Table 2: Matrix of responses

Exp Name	Y1	Y2	Y3	Y4	Y5
N1	5.032	58.54	16.949	88.01	2300
N2	9.969	72.51	14.810	61.49	4750
N3	9.401	74.26	13.647	57.63	1810
N4	5.478	54.80	9.689	32.81	645
N5	15.823	82.73	15.733	124.49	2624
N6	15.872	67.41	17.217	79.81	8904
N7	10.156	90.15	12.025	93.37	2639
N8	13.684	81.44	16.449	88.39	7790
N9	14.283	89.66	15.715	121.09	6934
N10	12.304	84.34	15.279	91.84	1710
N11	17.952	73.67	16.488	129.59	3254
N12	6.438	67.82	11.453	41.35	3459
N13	9.663	88.79	14.887	77.28	3974
N14	11.941	82.58	15.439	69.02	1120
N15	15.402	66.70	17.775	75.32	6000

Y_1 – Particle size (primary emulsion); Y_2 – Particle size polydispersion index (primary emulsion); Y_3 – Particle size (multiple emulsion); Y_4 – Particle size polydispersion index (multiple emulsion); Y_5 – Viscosity.

The validity of the experimental design was checked and the results obtained after data fitting shown that the results fit well all the responses.

Experimental design analysis.Formulation factor analysis. The influence of formulating factor on the studied responses is shown in fig.2.

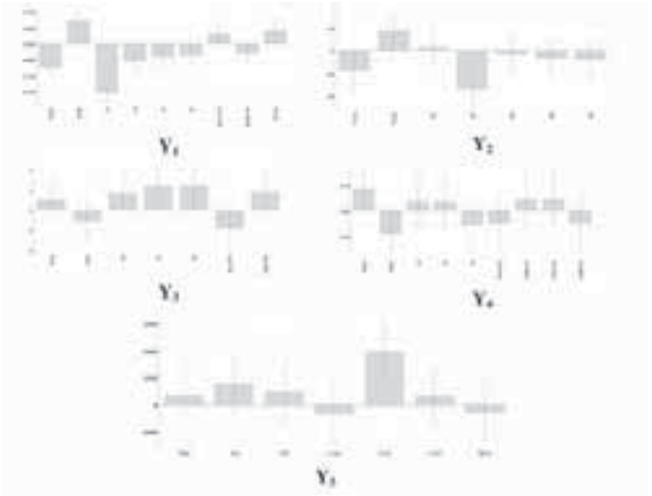

Figure 2. Influence of formulating factors on studied responses.

The mean diameter of the internal drop (Y1) is influenced by several factors. The most important is the stirring rate for the primary emulsion; when this increases the mean diameter of the drops decreases and then the type A homogenizer allows to obtain droplets with littlest mean diameter. The mean diameter of internal oil droplets is reduced also when the stirring time and stirring rate of secondary emulsion increases and when the concentration of hydrophilic surfactant increases. The homogeneity of the primary emulsion is influenced mostly by the stirring rate of the primary emulsion, this increases when the stirring rate is greater. Also the type A homogenizer allows to obtain the littlest polydispersion index. The mean diameter of the multiple emulsions is influenced by the ratio of lipophilic surfactant and the lipophylic phase ratio. The mean diameter of emulsion drops increases when these factors are greater. None of the studied factor has a significant influence on the polydispersion index of multiple emulsion drops. As to viscosity, this is significantly influenced only by the concentration of the lipophilic surfactant. The viscosity of multiple emulsion increases with the concentration of lipophilic surfactant.

CONCLUSIONS

This screening experiment allows setting the most important factors that influence the preparation of this O/W/O multiple emulsions.

References

1. De Luca M., Rocha-Filho P., Grossiord J.L. , Rabaron A., Vaution C., Seiller M. Les emulsions multiples. Int.J. Cosmet.Sci.13, 1-21 (1991).
2. Yanaki T. Preparation of 0/W/O type multiple emulsions and its application to Cosmetics. Studies in Surface Science and Catalysis 132 (2001) 1099 – 1014.

PO135

Development of chitosan gels for topical application of chlorhexidine hydrochloride

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INTRODUCTION

It is estimated that more then three quarters of the adult population wears dentures and many of them will later suffer from „denture stomatitis“ what is a recurring inflammation of the soft tissues supporting the denture. It is supposed that the denture stomatitis is caused by fungal infection, inappropriately fitting denture and insufficient oral hygiene. A promising, patient compliant treatment may be based on the using of a polymeric cushion with a gel-based tissue conditioner, which fits its form to the current state of the tissue.

In Slovak Republic is not registered any semisolid dosage form with chlorhexidine hydrochloride that is an efficient deficience and cationic antiseptic from the group of biguanid. Therefore it is commonly used in dermatology and stomatology. That is why we have commenced research aimed at the development of hydrogels on the basis of chitosan - a natural polysacharide that is biodegradable, biocompatible and bioadhesive. We have focused the attention to using urea in 1 and 2 % w/w. There were developed the hydrogels with chlorhexidine hydrochloride and investigated for in vitro release. The rheological properties were studied both with and without the drug.

MATERIAL AND METHODS

The chlorhexidine hydrochloride (CH) was obtained from Imperial Chemical Industries Ltd., (Great Britain), Chitosan (C) with medium molecular weight, Aldrich (Germany), lactic acid (LA) solution, citric acid (CA) and urea (U). All chemicals were obtained from Merck Chemical Company (Germany).

• Preparation of hydrogels

Chitosan gels were prepared at 2.5 w/w % concentrations in dilute lactic acid solution (2 w/w %) or citric acid solution (4 w/w %) in which was addied 0.1 w/w % chlorhexidine hydrochloride. Hydrogels were prepared without and with penetration enhancer – urea (1 or 2 w/w %).

• In vitro release of chlorhexidine from hydrogels

The release of drug from hydrogels was determined by using semipermeable membrane - Nadir Dialysierschlauch, Kalle A.G., Wiesbaden – Biebrich (Germany) on permeating apparatus – Faculty of Pharmacy Comenius University in Bratislava (Slovakia). The donor compartment was charged with 3.0 g of hydrogels. The receptor compartment was filled with 20 ml isotonic NaCl solution which was maintained at 37 ± 0.5 °C and stirred by magnetic bar. Amounts of the released drug were determined by a spectrophotometric method at 254 nm after 0.5 hr until 2 hrs and 3 hrs. (The sample taken from the receptor cell was replaced by equal volume receptor solution.) Blank hydrogels were used to determine the probable interaction of hydrogel base with the chlorhexidine hydrochloride absorbance.

• Evaluation of the rheological properties of hydrogels

Rheological parameters were determined at the temperature of 20 °C in the rheometer Viscotester VT 500 Haake Miss-Technik GmbH H.CO, Karlsruhe (Germany) with measuring system SV DIN. The results are averages of three paralel measurements.

RESULTS AND DISCUSSION

Rheological properties of chitosan hydrogels were evaluated. It is could be seen from the flow curve (Fig. 1) that hydrogels containing chlorhexidine hydrochloride exhibit pseudoplastic flow.

The addition of urea increased moderately the structural viscosity of the hydrogels without and with the drug. Deducing from the evaluation of the results obtained it may be concluded that all hydrogels have similar properties, which remained constant during their storing. Because the hydrogel rheograms without and with chlorhexidine hydrochloride were similar, no interaction between chitosan and drug is supposed.

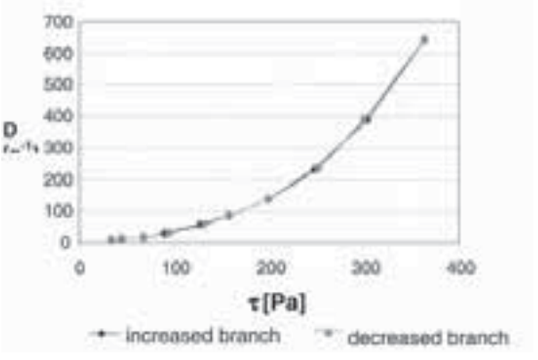

Figure 1: Rheogram of the chitosan hydrogel with chlorhexidine hydrochloride

In order to obtain a transparent adhesive chitosan hydrogel, the polymer was dispersed into solutions of acid. In the case of lactic acid the pH of hydrogel was 5.75 – 5.99. The pH of hydrogels with citric acid was 3.12 – 3.16. From the application point of view in dermatology and stomatology it is more suitable formulation with lactic acid with respect pH, which is weakly acid.

Release of chlorhexidine hydrochloride from the studied gels was monitoried through cumulative percentage of the released amounts.

The in vitro release studies showed that greather amounts of chlorhexidine hydrochloride were released from chitosan hydrogels with citric acid than from hydrogels with lactic acid. Differences are not statistically significant (P>0.05).

Presence of the enhancer – urea in the case of lactic acid caused an increase of the released drug (Fig. 2.), in the following order: 0 < 1 < 2 w/w % urea.

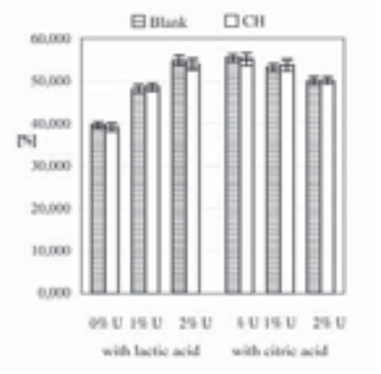

Figure 2: Released amounts of the drug from hydrogels after 3 hrs.

The greathest released amounts of chlorhexidine hydrochloride (54.89 %) was observed from the hydrogel of the following composition: 0.1 % CH + 2.5 % C + LA + 2 % U.

In the case of citric acid the presence of urea decreased the released amounts of the drug in the following order: 0 > 1 >2 w/w % urea. (Fig. 2)

The greathest released amount of chlorhexidine hydrochloride (55.49 %) was observed from the hydrogel of the following composition: 0.1 % CH + 2.5 % C + CA.

The release was evidently dependent on the presence of the urea and its concentrations.

The storring time did not significantly (P>0.05) influenced pharmaceutical availability of chlorhexidine hydrochloride from hydrogels.

CONCLUSION

With regard to the release, rheological properties and pH of the hydrogel on composition: 0.1 % chlorhexidine hydrochloride + 2.5 % chitosan + lactic acid + 2 % urea seams to be the most suitable.

ACKNOWLEDGEMENT

Research was supported by the Grant VEGA No. 1/0320/08

References

1. Varshosaz J., Jaffari F., Karimzadeh S. Development of bioadhesive chitosan gels for topical delivery of lidocaine. Sci. Pharm. 74: 209-223 (2006)
2. Kalász H., Antal I.: Drug excipients. Current Medicinal Chemistry. 13: 2535-2563 (2006)
3. Csóka G., Gelencsér A., Makó Á., Marton S., Zelko R., Klebovich I., Antal I.. Potential application of Metolose® in a thermoresponsive transdermal therapeutic system. Int J. Pharm. 338: 15-20 (2007)

PO136

Influencing the alaptide (spirocyclic dipeptide) liberation from hydrogels

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INTRODUCTION

In our study we focused on the improvement of the pharmaceutical availability of alaptide from assorted hydrogels containing glycerol as humectant, enhancer and preservative additive. Alaptide is a non-toxic spirocyclic dipeptide which, when administered dermally, has regenerative effects and improves the healing of a wounds, stimulate granulation tissue growth and accelerates the epithelisation process. It is a derivate of melanocyte-stimulating hormone-release inhibiting factor, which was by oral administration proved to influence some brain processes in animals including learning and memory[1,2].

Hydrogels were prepared from hydroxyethylcellulose (HEC 3% w/w) in distilled water, all with a 1% alaptide concentration (w/w). The composition of each of the hydrogels is presented in the legend of the Fig. 1. Glycerol is common used as an humectant and enhancer and also can prevent hydrogels from drying up and acts as a preservative additive. It was added into hydrogels with a 5%, 10% and 15% (w/w) concentrations. One kind of hydrogels was prepared without glycerol. The released amount of alaptide from each hydrogel kind was spectrophotometrically measured after 2., 7. and 14 days from preparation. All the experiments were performed *in vitro* with permeation apparatus. The timeframe of liberation experiment was 180 minutes, at this time was taking seven samples and measured up to 30 minutes. Based on the results was evaluated hydrogel with best liberation of alaptide.

EXPERIMENTAL METHODS

1. Materials

Alaptide - Cyclo[L-alanyl-1-amino-1-cyclopentanecarbonyl] in micronised form (VUFB-15754), Hydroxyethylcellulose (Hercules Incorporated), Glycerol (Interpharm s.r.o., SR), Sepicide HB® (Seppic ADINOP CO., LTD), Sodium chloride (Inerpharm s.r.o., SR), Cellophane membrane (EKOZ s.r.o., SR), Purified water (Faculty of Pharmacy, Comenius University, Bratislava, SR)

2. Instruments

Permeation apparatus - R&D Workshop of Faculty of Pharmacy, Comenius University, Bratislava, SR; Spectrophotometer–Philips Pye Unicam 8625 UV/VIS Ltd., (Cambridge, United Kingdom)

3. Drug release method

The release of alaptide was observed with seven continuous takings of samples during the experiment. 0.5 ml solution was taken from the donor chamber and was diluted with water. The absorbance of the sample was measured at λ = 220 nm within 30 minutes after the taking. Each chamber contained 3.0 g of gel or cream (on the donor side) and 20 ml of physiological solution (0.9% NaCl) on the acceptor side. The apparatus was warmed up and temperatured during the whole experiment at 37 °C ± 1°C.

RESULTS AND DISCUSSION

As we can see in the Graph, out of all hydrogels the least amount of alaptide, 34.21 %, was released from the 3% HEC hydrogel with 5% glycerol (w/w). The highest amount of released alaptide, 47.23 %, was measured from 3% HEC hydrogel containing 10% glycerol (w/w). Lower amounts were released from the following hydrogels: from 3% HEC hydrogel containing 15% glycerol (w/w), the released amount of alaptide was 36.44 %, from 3% HEC hydrogel without glycerol the released amount was 36.13%. We observed that alaptide was released better from hydrogels containing glycerol than from those not containing these substances, except 3% HEC hydrogel with 5% glycerol, where measured amount of released alaptide was less than by hydrogel without glycerol.

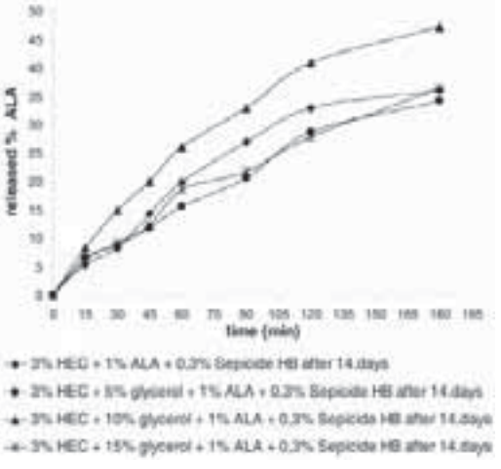

Figure 1: Time dependence of alaptide (ALA) releasing from hydrogels after 14. days

CONCLUSIONS

Not only the choice of the hydrogel basis but also the choice of additives from the humectants and enhancers class of substances is a factor influencing the liberation. The best results were obtained by using 10% of glycerol (w/w) in 3% HEC hydrogels. This knowledge can represent an important factor in the enhancement of the pharmaceutical availability of alaptide and its more effective utilization in therapy.

ACKNOWLEDGEMENT
This research was performed thanks to grants UK/104/2008 and UK/95/2008 from Comenius University and grant VEGA 1/0320/08.

References

1. Hliňák Z, Vinšová J, Kasafírek E. Effect of alaptide, its analogues and oxiracetam on memory for anelevated plus-maze in mice. Eur. J. Pharmacol. 314: 1-7 (1996).
2. Lebedev A. A, Panchenko G. N. Shabanov P. D. Effect of the melanostatin analogue alaptide on dopamine- dependent behavior in rats reared in isolation. Zh. vysshei nervnoi deiatelnosti imeni IP Pavlova 50: 716-719 (2000).

PO137

In vitro adhesive properties and release kinetics for mucoadhesive gel systems containing metronidazol

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INTRODUCTION

Among the antibiotics that have been considered for periodontal treatment, metronidazole has often been chosen because of its selective efficacy against obligate anaerobes. Conventional methods and preparations in treatment of the periodontal disease do not exert sufficient efficacy due to inaccessibility of the anaerobic bacteria dwelling in the periodontal pockets. Local delivery devices were widely studied for various applications. Fibers, films, strips and microparticles made of polymers have reported as effective methods to administer antibacterial agents for periodontal therapy. Together with these solid devices, semisolid adhesive formulations have also been proposed [1,2]. Mucoadhesive drug delivery systems would offer several advantage over currently available systems, including ease of application into and good retention within the periodontal pocket. Some mucoadhesive polymers (hydroxyethyl cellulose-HEC, Carbopol 934P and 974P, polycarbophil-Noveon AA-1, Pemulen TR-1) have been studied as components of delivery systems for gels containing 5 or 25 % suspended metronidazole [2].

In our former studies, using Carbopol 940NF, Carbopol 974P and Ultrez 10 as hydrogel bases, we formulated, prepared and rheologicaly characterized three mucoadhesive types of gels containing 15% metronidazole. Each of these gels were obtained in three variants by using sodium hydroxide, sodium carbonate, triethanolamine respectively as neutralizing agents. The aim of this study was the comparatively in vitro evaluation of mucoadhesive qualities and release kinetics for these nine metronidazole-containing gel systems.

EXPERIMENTAL METHODS

• Materials and products

Metronidazole; Carbopol 940 NF (C940), Carbopol 974P NF (C974), Ultrez 10 (U10) (BF Goodrich, USA), sodium hydroxide (NaOH) (Chemapol Praha Czechoslovachia), sodium carbonate ($NaCO_3$), triethanolamine (TEA) (Merck).

The nine bioadhesive gels were prepared using 1% aqueous polymer (Carbopol 940, Carbopol 974, Ultrez 10, respectively); agents for achieve neutrality pH range 6,5-7,0 at 25 ^{0}C (NaOH, $NaCO_3$, TEA, respectively); 15% suspended metronidazole.

• Measurement of adhesion qualities

Conditions: Analyzed samples were introduced into a tubular cell (13 mm diameter, 8 cm height and 4 mm thickness) provided with a synthetic semi-permeable membrane. The balance system of apparatus determined the mass needful for detach the samples from membrane of the cell.

The detachment force was calculated using the following formula: F= G x g (F= adhesion force, in newton-N; G= necessary mass for determine detachment of the gel from the surface of the semi-permeable membrane, in kg; g= 9,81 m/s^2).

• In vitro release of metronidazole

Apparatus: Pharma Test PTWS (paddle stirrers); Conditions: 5 g metronidazole-containing gel (donor phase); 100 ml water at 37 ^{0}C (acceptor phase); 20 rpm; times: 5, 10, 15, 30, 45, 60 min; amount of metronidazole dissolved was determined by employing UV absorbtion at the wavelength of maximum absorbance at about 320 nm.
Calculations:

$$k = \frac{l}{T} \times \ln \frac{100}{100 - C}$$

k= release constant (sec^{-1})
C= concentration at T time
T= time of prelevation.

RESULTS AND DISCUSSION

As it is obvious in the Table 1 and Figure 1, the metronidazole presence in the gel determine a lightly decreases of adhesive properties of the gel aqueous system formed by the three used polymers. Adhesivity is also influenced by neutralizing agent used: Carbopol 974-$NaCO_3$ and Ultrez 10-NaOH gels have upper adhesive qualities.

Table 1: In- vitro adhesive test results

Gel		Without metronidazole		With metronidazole	
		G (g)	F (N)	G (g)	F (N)
C 940	NaOH	24	0,235	20,0	0,196
	Na_2CO_3	25	0,245	20,7	0,203
	TEA	26	0,255	18,3	0,180
C 974	NaOH	29	0,284	25,0	0,245
	Na_2CO_3	31	0,289	26,7	0,262
	TEA	30	0,304	21,7	0,213
U 10	NaOH	30	0,294	24,3	0,239
	Na_2CO_3	30	0,294	23,0	0,226
	TEA	32	0,313	22,3	0,219

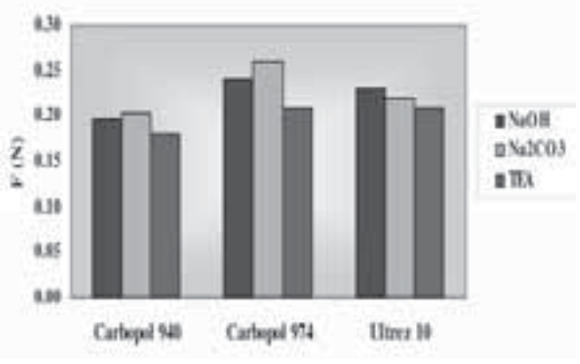

Figure 1: Adhesion qualities of the gels containing 15% metronidazole

Experimental results obtained by testing the metronidazole release from structure of the system gels shown that 7,83-19,30% from initial active substance content of the samples were dissolved in water after 60 minutes, the constants of the release speeds being specified in table 2.

Table 2: Metronidazole release constants (k)

Gels containing 15% metronidazole		Equation of release regression curve (R^2)	k (min^{-1}) ± SD
C 940	NaOH	y=38,80x-24,06 (0,9766)	0,0034 ± 0,0011
	Na_2CO_3	y=83,26x-34,55 (0,9895)	0,0035 ± 0,0012
	TEA	y=56,66x-25,13 (0,9837)	0,0036 ± 0,0011
C 974	NaOH	y=76,23x-28,38 (0,9893)	0,0035 ± 0,0010
	Na_2CO_3	y=72,07x-22,49 (0,9944)	0,0034 ± 0,0008
	TEA	y=72,33x-20,29 (0,9927)	0,0034 ± 0,0009
U 10	NaOH	y=81,33x-22,17 (0,9942)	0,0035 ± 0,0010
	Na_2CO_3	y=70,27x-20,61 (0,9943)	0,0034 ± 0,0008
	TEA	y=74,37x-25,98 (0,9766)	0,0033 ± 0,0011

CONCLUSION

The carboxyvinil polymer type influences the adhesive properties of the studied mucoadhesive system-gels containing metronidazole. Carbopol 974 gel neutralized with sodium carbonate has the better adhesivity. The alkaline substances used for neutralizing the gels have less influence.

After 1 hour, the released metronidazole quantities from the nine studied gels are between 7,83-19,3% from initial content of the gels. In the cases of Carbopol 974 and Ultrez 10 gels, the neutralizing agent used has no influence on the drug release, at about 17% metronidazole released.

References

1. Schwach-Abdellaoni K., Vivien-Castioni N., Gurny R. Local delivery of antimicrobial agents for the treatment of periodontal diseases. European Journal of Pharmaceutics and Biopharmaceutics 50: 83-99 (2000).
2. Jovanovic I., Toskic Radojicic M., Nonkovic Z. Formulation of mucoadhesive dental gels containing metronidazole for application to periodontal pockets. Proc. 2nd World Meeting APGI/APV, Paris: 1005-1006 (1998).

PO138

Bioadhesive gel formulations of econazole nitrate with chitosan for the treatment of vaginal candidiosis

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INTRODUCTION

The use of bioadhesive polymers as carriers is supposed to ensure prolonged intravaginal residence time and to develop suitable drug delivery. Econazole nitrate (EN) is used mainly for the vaginal infections and diseases caused by both candidas or other fungi and Gram-positive bacteria. Chitosan (CH) is mostly obtained by deacetylation of chitin derived from the exoskeleton of crustaceans and it provides an extended retention time due to its strong bioadhesive characteristics. CH has valuable properties as a biomaterial because it is considered to be biocompatible, biodegradable and non-toxic. The cationic character and potential functional groups of chitosan make it an attractive biopolymer for many biomedical and pharmaceutical applications. It is a promising bioadhesive material at physiological pHs and its gels as vehicles are also suitable for poorly soluble drugs [1].

In our study, we prepared vaginal bioadhesive gel formulations of EN with CH in different molecular weights for the treatment of vaginal candidiosis. Formulations which have been designed for vaginal application

must exhibit acceptable mechanical characteristics e.g. ease of application, low hardness and good retention at the site of application. Vaginal gels were evaluated according to their mechanical characteristics, mucoadhesive properties, sol/gel transition temperature, viscosity, and release behaviors.

EXPERIMENTAL METHODS

• **Preparation of Bioadhesive Gels:** The gel formulations were prepared using CH base with three different molecular weights at 2% (w/w) concentration in diluted lactic acid solution (1%). EN was incorporated into the formulations at 1% (w/w) concentration. The composition of gels is given in Table 1.

Table 1: The composition of formulations

Codes	CH_{low} %	CH_{medium} %	CH_{high} %	EN %
F1	2	-	-	
F2	-	2	-	
F3	-	-	2	
F4	2	-	-	1
F5	-	2	-	1
F6	-	-	2	1

• TPA Studies

Textural analysis of gel formulations were performed using Software-controlled penetrometer [TA-TX Plus, Stable Micro System, UK] equipped with 5 kg load cell in texture profile analysis (TPA) mode. In this, an analytical probe was twice inserted into each formulation to a defined depth (15 mm) and defined rates (2 mm/s), allowing a delay period (15 s) between the end of the first and beginning of the second insertion. From the resultant force-time curve mechanical parameters (hardness, compressibility, adhesiveness and cohesiveness) were derived.

• pH Measurements

To investigate the compatibility of the formulations for vaginal application, their pH values were measured by a pHmeter (NEL Mod.821) at room temperature and were also checked after 24 hours.

• Viscosity Measurements

Viscosity measurements of the gels were performed on a Brookfield digital viscosimeter (Model DV-III) at 25 ± 1°C.

• In Vitro Release Studies

The in-vitro release of EN from different gel bases was carried out using Spectra/Por Regenerated Cellulose Dialysis Membrane. Phosphate buffer (pH 7.4) with dioxane (65:35 v/v) was used as the receptor medium. 6 g of each formulation was packed in dialysis bags which were placed in 150 ml of buffer at 37 ± 0.5°C in sealed glass vials. At certain time intervals, samples were withdrawn and the content of EN in each sample was analyzed with Shimadzu UV-VIS 1208 spectrophotometer. All the experiments were repeated three times.

RESULTS AND DISCUSSION

Vaginal semi-solid formulations should have appropriate mechanical properties for the maximum benefit of the patient from the formulation.

The mechanical properties of bioadhesive gels of EN are presented in Table 2. The results revealed that, higher molecular weight of CH led to a higher hardness and adhesiveness. The compressibility of the gels also increased when the molecular weight increased. However, it was seen that the absence of EN doesn't have a significant effect on the mechanical strength of vaginal gel formulations.

Table 2: Mechanical properties of gels (mean and standard deviation were calculated for n=5)

Codes	H (N) ± SD	A (N.mm) ± SD	Coh ± SD	Comp (N.mm) ± SD	E ± SD
F1	0.008 ± 0.000	0.007 ± 0.000	0.983 ± 0.006	0.014 ± 0.000	1.057 ± 0.235
F2	0.012 ± 0.002	0.031 ± 0.007	0.957 ± 0.012	0.014 ± 0.003	0.964 ± 0.078
F3	0.016 ± 0.000	0.095 ± 0.004	1.050 ± 0.027	0.020 ± 0.000	1.205 ± 0.100
F4	0.007 ± 0.000	0.006 ± 0.000	0.990 ± 0.052	0.012 ± 0.001	1.004 ± 0.052
F5	0.013 ± 0.001	0.029 ± 0.014	0.921 ± 0.105	0.017 ± 0.001	0.981 ± 0.069
F6	0.036 ± 0.012	0.090 ± 0.010	0.978 ± 0.046	0.036 ± 0.009	1.154 ± 0.013

(H: Hardness, A: Adhesiveness, Coh: Cohesiveness, Comp: Compressibility, E: Elasticity)

pH values of all the prepared CH gels were with in vaginal pH limitations and suitable for vaginal application. pH values of all the prepared chitosan gels were given in Figure 1.

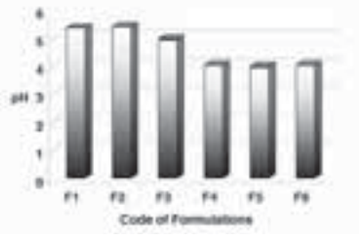

Figure 1: pH values of chitosan gels

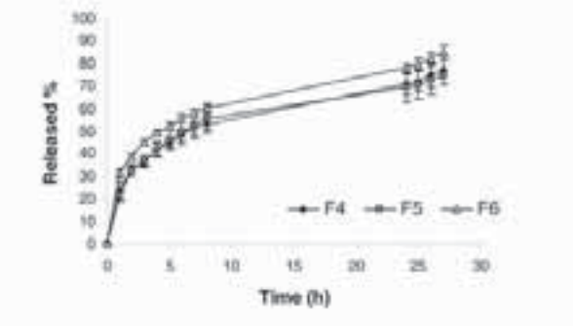

Figure 2: The release profiles of chitosan gels of EN

All the gel formulations exhibited pseudoplastic behaviour as was expected.

Usually, it is expected that the release rate becomes slower with the increasing molecular weight of CH [2]. However, different observations can occasionally be obtained [3]. Our study showed that the molecular weight of CH did not significantly affect the release rate (Fig. 2). Gel prepared with medium molecular weight CH also showed a similar release profile with the other formulations.

CONCLUSION

Vulvavaginal candidiosis is a common problem worldwide, affecting all strata of society. There has been a growing tendency to use shorter courses of topical agents. EN, is an important antifungal agent which is effective for the local treatment of vaginitis.

In our study, vaginal bioadhesive gel formulations of EN were prepared using chitosan with three different molecular weights. TPA, pH, viscosity and release studies of the preparations were carried out and any significant difference was not observed. It was shown that, chitosan with different molecular weights can be used as a bioadhesive polymer to prepare the gel formulations of EN for vaginal application.

References

1. Illium L., Chitosan and its use as a pharmaceutical excipient, Pharmaceutical Research, 15(9), 1326-1331, 1998.
2. Hui Yun Zhou, Xi Guang Chen, Ming Kong, Cheng Sheng Liu , Dong Su Cha, John F. Kennedy, Carbohydrate Polymers, 73, 265–273, 2008.
3. Aksungur P., Sungur A., Ünal S., skit A. B., Squier C.A.,[a]enel S., Chitosan delivery systems for the treatment of oral mucositis: in vitro and in vivo studies, Journal of Controlled Release, 98,269-279, 2004.

This project was supported by TUBITAK-Research Grant.

PO139

The influence of the temperature on the conductivity of polyNIPAM microgels for controlled release of drugs

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INTRODUCTION

The discrete microgels are colloidally stable crosslinked polymeric networks on the size scale of nanometers to micrometers. The deswelling process is controlled by diffusion, where the rate of the collapsing of the macromolecule is correlated to the dimensions of pores in the polymeric matrix. In the swollen state the microgels may contain approximately up to 95% of water, whereas in the deswollen state there is only 20% of water in the polymeric matrix. PolyN-isopropylacrylamide (polyNIPAM) is one of the most utilised macromolecules, characterized by reversible volume phase transition at 31 °C. Characterization of polyNIPAM microgels can be performed using different methods, i.a. dynamic light scaterring, differential scanning calorimetry, or neutron scattering. The microgel thermosensitive particles may be applied in many medical devices, including drug forms for topical use. With the collapsing and the expanding of the macromolecule in the aqueous eviroment the molecules of biologically active substance may be released in the controlled manner [1]. During the synthesis of microgel particles with different co-monomers the macromolecules of required characteristics are obtained, e.g. anionic, cationic or hydrophobic polyNIPAM polymers [2]. The conductivity of this systems could be a source of data, regarding the delivery of anionic and cationic drug molecules to the skin surface.

The aim of the present study was to investigate the conductivity of polymeric beads containing modified polyNIPAM polymers, at temperatures below and over the VPPTs (Volume Phase Transition Temperatures), as well as in the temperature of the human skin surface, for the development of topically applied drug forms.

EXPERIMENTAL METHODS

Poly(NIPAM) microgels particles were synthesized by surfactant free emulsion polymerization in deionized water at 343 K, under an inert nitrogen atmosphere. The polyplyNIPAM, the polyNIPAM-co-butyl acrylate and the polyNIPAM-co-itaconic acid were synthesized. The ATR-FTIR spectroscopy was used to analyze reagents and formed microgels in order to find differences in molecular structures among microgels with different composition, and to confirm the reaction course. The VPPTs of synthesized microgels were assessed by the turbidimetric method in the temperature range between 18 and 45 °C in the diluted samples – the 2100 Hach Turbidimeter was used. The conductivity measurements were performed with Multi Seven Toledo Metler conductometer. Every measurement was repeated five times, and the ANOVA statistical analysis was performed, to elucidate the differences in and between the groups of samples. The samples of synthesized microparticles were purified by dialysis in deionised water up to constant conductivity, not higher than 25 mcS/cm in the temp. of 25 °C.

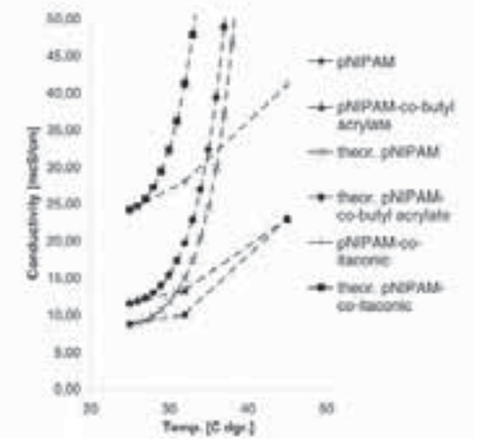
RESULTS AND DISSCUSSION

The analysis of the ATR-FTIR specters of NIPAM monomer and synthesized molecules of polyNIPAM confirmed that inter and intramolecular bonds were created and the reaction of the polymerization was documented by the changes in stretching vibrations of N-H groups and amide I and amide II bands.

The VPPT of the assesed microgels was in the range of 31 – 32 °C in the case of polyNIPAM and the polyNIPAM-co-butylacrylate, whereas in the case of the polyNIPAM-co-itaconic acid only slight turbidity was observed in the temperatures over 43 °C. The temperature range for the VPT was presented in the table.

Polymer	Temperature of VPT	
	Initial	Final
polyNIPAM	31	40
polyNIPAM-co-butyl acrylate	29	37
polyNIPAM-co-itaconic acid	43	Over 45

The assessed conductivities were in the renge between 8,73 and 41,12 mcS/cm. The highest values were obtained in the case of polyNIPAM-co-itaconic acid, which can be explained as a consequence of high content of anionic groups present in the macromolecule.



The influence of the temperature on the conductivity of synthetisized microgel dispersions

The polyNIPAM and polyNIPAM-co-butyl acrylate also are characterised as anionic polymers according to the initiatior used during the synthesis. Although the content of the anionic groups, which are important for the dissociation of water molecules, is lower than in the case of bicarboxylic itaconic acid comonomer. The assessed values of the conductivities for the synthetised polymers are compared to the estimated values of conductivity in the aqueos enviroment, assuming that the slope of conductivity increase is in the range of 2% for every 1 °C of the temperature increase. The changes of the conductivity, according to the temperature increase, below and over the VPPT, are presented on the graph.

The polyNIPAM - and the more hydrophobic polyNIPAM-co-butyl acrylate - were characterised by similar conductivities in the assessements conditions, i.e. between 8,73 and 22,98 mcS/cm.

CONCLUSION

The conductivity measurements may be applied as an additional direction for the nature and behaviour of the new synthesized microparticles. The influence of the temperature on the conductivity of microparticles dispersions, considering the specified VPPT of microgels will be evaluated, as it would have an impact on the loading and release of biologically active substances.

This research was supported by a Marie Curie Transfer of Knowledge Fellowship of the EC 6th FP under contract no. MTKD-CT-2005-029540-POLYSURF.

References

1. Gan D., Lyon L. A., Synthesis and protein adsorption resistance of PEG-modified poly(N-isopropylacrylamide) core/shell microgels, Macromolecules 35: 9634, 2002
2. Castro Lopez V., Hadgraft J., Snowden M.J., The use of colloidal microgels as a (trans)dermal drug delivery system, Int. J. Pharm. 292: 137–147, 2005

PO140

Development of in situ-gelling and thermosensitive ketoprofen liquid suppositories

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INTRODUCTION

In situ-gelling systems are liquid aqueous solutions before administration, but gel under physiological conditions and these do not require organic solvents or copolymerization agents [1]. Poloxamer® series are the most commonly encountered thermosensitive systems in the pharmaceutical field. Liquid suppositories are usually prepared from poloxamers since an aqueous solution of poloxamers, at high concentration, exhibits reversible thermal gelation [2]. Liquid suppositories are converted to semisolids in the rectum by thermal gelation following rectal administration. Ketoprofen (KP), which is highly potent and safe nonsteroidal drug of the propionic acid derivate group, has antiinflammatory, analgesic and antipyretic effects in treatment. Since KP produces gastro-intestinal side effects, its administration rectally is considered as a serious alternative to the oral route. The aim of this study is to develop thermosensitive and mucoadhesive liquid suppositories containing ketoprofen using poloxamer and different bioadhesive polymers.

EXPERIMENTAL METHODS

• Preparation of liquid suppositories

Ketoprofen and various amounts of different bioadhesive polymers (PVP, CMC, HPMC and Carbopol 934 P) were completely dispersed in distilled water with continuous agitation at room temperature and cooled down to 4°C. The mixture of poloxamer 407 and poloxamer 188 was then slowly added to the solution with continuous agitation. The liquid suppository was left at 4°C through the night until a clear solution was obtained. The composition of the formulations is listed in Table 1.

• Measurement of gelation temperature

A 20-ml transparent vial containing a magnetic bar and 5 g of poloxamer solution was placed in a low temperature thermostat water bath. A digital thermosensor connected to a thermistor was immersed in the poloxamer solution. Poloxamer solution was heated at a constant rate (1ºC/min) with constant stirring (130 rpm). The gelation temperature was determined as the temperature registered on the thermistor, when the magnetic bar stopped moving due to gelation [2]

Table 1: Composition of liquid suppository formulations

F	P407/ P188 %4/%20	K %2.5	C (%)	HPMC (%)	CMC (%)	PVP (%)
F	+	-	-	-	-	-
F1	+	+	-	-	-	-
F2	+	+	0.2	-	-	-
F3	+	+	0.4	-	-	-
F4	+	+	0.6	-	-	-
F5	+	+	0.8	-	-	-
F6	+	+	1.6	-	-	-
F7	+	+	-	0.2	-	-
F8	+	+	-	0.4	-	-
F9	+	+	-	0.6	-	-
F10	+	+	-	0.8	-	-
F11	+	+	-	1.6	-	-
F12	+	+	-	-	0.2	-
F13	+	+	-	-	0.4	-
F14	+	+	-	-	0.6	-
F15	+	+	-	-	0.8	-
F16	+	+	-	-	1.6	-
F17	+	+	-	-	-	0.2
F18	+	+	-	-	-	0.4
F19	+	+	-	-	-	0.6
F20	+	+	-	-	-	0.8
F21	+	+	-	-	-	1.6

(K: Ketoprofen; C: Carbopol 934 P)

• In vitro drug release from liquid suppositories

In vitro drug release of ketoprofen from liquid suppositories was monitored by the USP XXVI paddle method at a rotating speed of 100 rpm in 500 mL phosphate buffer, pH 7.2 at 37 ± 0.5°C. Five grams of each formulation containing 125 mg of ketoprofen was inserted into a semipermeable membrane tube (Spectra/por® 1 Dialysis Membrane). Both sides of the tube were tied up with a thread to prevent leakage. The semipermeable membrane tube was then immersed in the dissolution medium. In the experiments, a 0.5 mL sample was withdrawn from dissolution medium at selected times and an equal volume of medium was returned to the system after withdrawal. The samples were then assayed spectrophotometrically at 261nm.

RESULTS AND DISCUSSION

The gelation temperature of liquid suppositories was dependent on the concentration of poloxamers (P407 and P188) and mucoadhesive polymers and also the addition of drug. It was seen that the decrease of the concentration of P407 from 10 % to 4 % resulted in an increase of gelation temperature from 48ºC to 64ºC. The addition of ketoprofen significantly decreased the gelation temperature. In the P407/P188 mixtures, as the content of P407 changed from 10 to 4% a gelation temperature decreasing effect of ketoprofen was consistently observed (Table 2).

Table 2. Effect of KP on the gelation temperature of poloxamer systems (n=3)

P407/P188 (%)	Ketoprofen (%)	Gelation Temperature (ºC)
10/20	0	48
10/20	2.5	33.5
8/20	0	52
8/20	2.5	34.1
6/20	0	58
6/20	2.5	35.5
4/20	0	64
4/20	2.5	37.1

The effects of bioadhesive polymers were also determined. It was seen that the decrease of gelation temperature was higher in the formulations prepared with Carbopol than the other bioadhesive polymers (data not shown). Among the preformulations of P407/P188/KP mixtures, 4/20/2.5% was selected as the suitable system for preparing of liquid suppository.

As to the obtained results of in vitro drug release studies, Carbopol has significant effect on release rate among the bioadhesive polymers. It was seen that the release rate decreased with increasing of Carbopol cocentration (Fig. 1). The decrease of release rate for the formulations containing HPMC, CMC and PVP in the concentration of 1.6% was 11, 8 and 5 % respectively during 8 hours. However, the concentration increases of HPMC, CMC and PVP had no effect on release rate (data not shown).

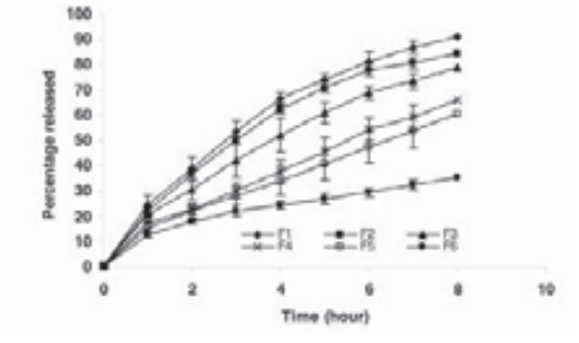

Figure 1: Release of ketoprofen from liquid suppository formulations containing Carbopol in different concentrations (n=3)

CONCLUSION

Liquid suppository formulations of ketoprofen were successfully prepared using P407, P 188 and different types of bioadhesive polymers. According to the obtained results, the liquid suppository formulations prepared with poloxamer systems and Carbopol were found to be promising formulations as new anti-inflammatory dosage form for effective therapy.

References

1. Ruel-Gariepy E., Leroux JC. In situ-forming hydrogels-review of temperature-sensitive systems. Eur. J. Pharm. Biopharm. 58: 409-426 (2004).
2. Choi HG. et al. Development of in-situ gelling and mucoadhesive acetaminophen liquid suppository. Int. J. Pharm. 165: 33-44 (1998)

PO141

Development and evaluation of low dose proniosomal gel for delivery of celecoxib

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INTRODUCTION

Osteo-arthritis is the most common of rheumatic diseases and is the principal source of pain and disability in the elderly (1). NSAIDs (Non Steroidal Anti- inflammatory Drugs) are the drugs of choice for the management of these degenerative orthopathies (2). The major drawback to anti- inflammatory drug use is the preponderance of gastrointestinal side effects (GI perforations, ulcerations and bleeding) encountered with majority of agents, when given orally. The NSAID mediated toxicity is often dose related (3). Thus reduction in serum concentration should also lessen the risk of potentially serious systemic adverse effects. This originates the need of an alternative route of administration, which can bypass the gastro-hepatic metabolism of the drug. Transdermal route is an alternative choice of route of administration for such drugs.

The aim of the present study was to develop a low dose transdermal drug delivery system (proniosomal gel) for celecoxib (NSAID), which undergo hepatic first- pass metabolism and show low bioavailability. Celecoxib possesses all the characteristics that make it an ideal candidate for developing a transdermal delivery system; high partition coefficient (Log P - 3.683) (4), low molecular weight (MW - 381.38), low bioavailability (22 – 40%) (5), when administered orally causes serious gastrointestinal events like bleeding, ulceration, and perforation of the stomach, small intestine or large intestine.

PREPARATION OF PRONIOSOME GEL (6)

Proniosomal gel was prepared by taking surfactant (Span 40 and 60), alcohol (ethanol or isopropyl alcohol) (1:1), and drug (100 mg) in a clean and dry glass tube. All the ingredients were mixed in the tube. The open end of the glass tube was covered with a lid to prevent the loss of solvent from it and warmed on a water bath at 60-70°C until the surfactant dissolved completely. The aqueous phase (isotonic phosphate buffer of pH 7.4) was then added and warmed on the water bath till clear solution was formed which on cooling got converted into a proniosomal gel. The ultimate ratio of surfactant: alcohol: aqueous phase was 5:5:4 w/w/w. The gel obtained was preserved in same glass tube in dark for characterization.

EVALUATION

The prepared proniosomal gel was evaluated for size and size distribution, microscopic study, entrapment efficiency, *in-vitro* skin permeation study, stability and pharmacodynamic studies. The drug release and skin permeation studies were analyzed by one-way analysis of variance (ANOVA). A Dunette multiple comparison tests and paired t-test were used to compare different formulations and a p-value of less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Proniosomes were prepared based on span-40 and span-60. The % entrapment showed a high value (> 90%). The vesicle shape was determined with the help of transmission electron microscopy (TEM) and the niosomes were found to be spherical (Fig. 1). The vesicle size, size-distribution (257 – 816 nm) and polydispersity (0.084 - 1.38) studies were performed using photon correlation spectroscopy. Permeation of

Table 2: Composition and appearance of different proniosomal formulations (Drug =100 mg)

Formulation code	Ingredients					Appearance
	Span (mg)	Cholesterol (mg)	Soya Lecithin (mg)	Alcohol (ml)	Aqueous Phase (IPB)* (ml)	
N1LE2	1800	200	1800	2.5	1.6	Translucent amber gel
N1LE3	1800	200	900	2.5	1.6	Translucent amber gel
N2LE3	1800	200	900	2.5	1.6	Translucent amber gel

N1 = Span 40, N2 = Span 60, E = Ethanol, *IPB = Isotonic phosphate buffer (pH 7.4)

celecoxib across excised rat skin from various proniosomal gel formulations was investigated and % cumulative permeated (N1LE2 - 79.161%, N1LE3 - 86.262%, N2LE3 - 60.614%, Niosome suspension - 80.3%) and flux (mg/cm^2/h) (N1LE2 – 0.1503, N1LE3 – 0.1736, N2LE3 – 0.1188, Niosome suspension – 0.0645) were determined (Fig. 2). *In vivo* (anti-inflammatory) studies were performed by carrageenan (1% w/v) induced rat hind-paw edema method. The selected proniosomal gel (N1LE3) produced 100% inhibition of paw edema in rats up to 8h after carrageenan injection and then it was 95.26 % and 91.71 % inhibition after 12 h and 24 h respectively (Fig. 3). The stability studies were performed at 4°C and at room temperature and all the formulations were found to be stable.

CONCLUSION

Proniosomes of a choice of compositions have been studied and evaluated. Proniosomes may be a hopeful carrier for celecoxib and other drugs, especially due to their simple production and be deficient in unnecessary use of pharmaceutically unacceptable additives.

Thus, based on above studies and discussions, it was found that formulation N1LE3 has the best effective combination of surfactant, alcohol and aqueous phase among the formulations studied for further development of proniosomal gel for the transdermal delivery of celecoxib for the treatment of osteoarthritis. From this study it can be concluded that celecoxib can be formulated into a low- dose proniosomal gel for transdermal delivery that can save the recipient from the harms of large doses with improved bioavailability (by by-passing the hepatic first metabolism) and can be recommended for further pharmacokinetic and pharmacodynamic studies in suitable animal models.

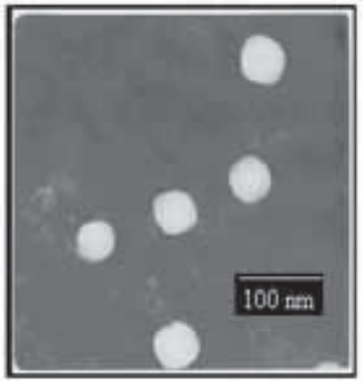

Figure 1: TEM images of N1LE3 niosomes showing spherical shapes

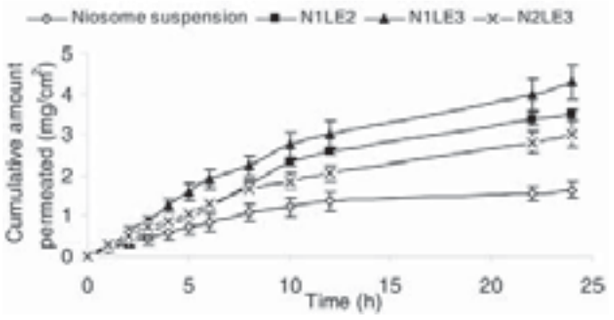

Figure 2: Drug permeation profile of proniosomal formulations (N1LE2, N1LE3, N2LE3) and niosome suspension

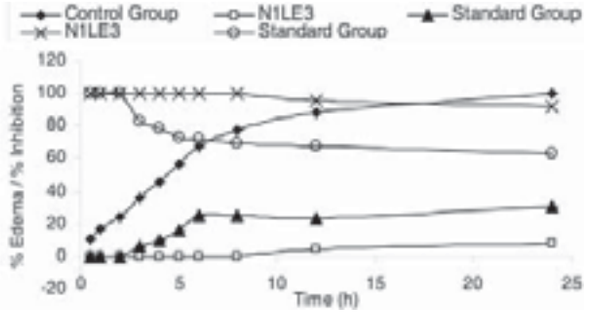

Figure 3: In-vivo studies of proniosomal gel

References:

1. Arthritis Research Campaign. Arthritis: the big picture. http://www.arc.org.uk/about_arth/bigpic.htm (accessed 17 May 2004).
2. Kean Walter, F., Buchanan, W. Watson, 2005. The use of NSAIDs in rhumatic disorders 2005: a global perspective, Inflammopharmacology. 13 (4), 343 – 370.
3. Griffin, M. R., Scheiman, J. M., 2001. Prospects for changing the burden of nonsteroidal anti-inflammatory drug toxicity. Amer. J. Med. 110 (1), S33 – S34.
4. Diwan, P. V., Reddy, M. N., Rehana, T., Ramakrishna, S., Chowdary, K. P. R., 2004. -Cyclodextrin Complexes of Celecoxib: Molecular-Modeling, Characterization, and Dissolution Studies. AAPS PharmSci. 6 (1), Article 7.
5. Susan, K. P., Margaret, B.V., Susan M.J., Lawal Y., 2001. Pharmacokinetics of Celecoxib after oral administration in Dogs and Humans. J. Pharm. Exp. Therap. 291 (2), 638-645.
6. Vora, B., Khopade, A. J., Jain, N. K., 1998. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J. Cont. Release. 54, 149-165.

PO142

The promising emulsion formulations of ostrich oil produced from pre-fleshing wastes of ostrich skin

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INTRODUCTION

There are three major species of birds from the ratite family namely ostrich, emu and rhea. Both the emu and ostrich are within the same family, called Struthionidae. These birds are produced primarily for their meat, leather, and oil [1]. The main market for ostrich oil is cosmetic preparations such as, moisturizing creams, body lotion, soap, and lip balm. Ostrich oil has been also used in the treatment of lesions, burns, contact dermatitis, eczema, psoriasis, dry skin, dry hair conditions, bed sores, and many other ailments [2]. The objective of this study was to prepare different emulsion formulations with ostrich oil produced from pre-fleshing wastes of ostrich skins for topical applications.

EXPERIMENTAL METHODS

• Producing of Ostrich Oil

Pre-fleshing wastes obtained from Ostrich (Struthio camelus camelus) skin were used, after soaking process. Firstly, these wastes that contain natural fats of Ostrich skin have been cleaned with water by washing at medium temperature for eliminating blood, dirt, skin, etc. Then, these fatty wastes have put into the reaction vessel and cooked under vacuum to reduce time under heat to avoid damage to final product at 50°C ± 5°C by using basket heater for 2 hours. After that, this phase was decanted to separate the solids and to remove pollutants by filtration. Then, fatty phase was centrifuged to coagulate processed fat in the mix 4°C [3]. Figure 1 showed the flow chart of ostrich oil production.

Figure 1: Flow chart of ostrich oil production

• Preparation of Formulations

O/W (F1), W/O (F2) and F3 (O/W) emulsion formulations were prepared by slow addition of the internal phase to the external phase under continuous mechanical stirring (IKA-WERCK RV20DZM). Ostrich oil was used as oil phase for all formulations. W/O/W (F4) multiple emulsion formulation was prepared by a two-step emulsification process In the first step primary W/O emulsion was prepared by slowly adding water phase to the oil phase. The system was stirred at 2000 rpm for 20 minutes. In the second step, the primary emulsion was added slowly to mixture hydrophilic phase at 800 rpm for 30 minutes [4]. The compositions of formulations were shown in Table 1.

• Characterization of Formulations

After preparation of emulsions, microscopic observations were realized with an optical microscope. Conductivity of formulations was measured in order to determine the emulsion type. The pH values of the emulsion formulations were measured by a pH meter (NEL Mod.821) at 25 ± 1°C.

Table 1: Composition of emulsion formulations (w/w)

	F1	F2	F3	F4
Ostrich oil	20	20	20	19.2
Abil EM 90				3.2
Magnezyum sulphate				0.56
Carbapol 940	0.375		5	
Tween ® 80	3.25			
Span ® 80	1.75	5	0.375	
Trietanolamin	0.3		0.3	
Synperonic			2.5	0.8
Distilled water	74.325	75	71.825	76.24

• Stability Tests

The samples of freshly prepared emulsions were subjected to the centrifugal stress for measuring the physical stability of the emulsions. Stability of formulations was also tested at 8 ± 1°C, 25 ± 1°C and 40 ± 1°C during three months.

RESULTS AND DISCUSSION

Ostrich oil which obtained from pre-fleshing wastes of ostrich skin was used to prepare emulsion formulations. Emulsions were characterized macroscopically and microscopically. Macroscopically, the emulsions were white and homogenous creams; their appearance was checked every day for signs of phase separation or microbial contamination. Microscopic observations clearly demonstrated emulsion types. It was confirmed by conductivity analysis, too. According to the results of stability tests, a phase separation was observed in F2 and F4 formulations at 8 °C, 25 °C and 40 °C. Because of that the data belonged to F2 and F4 didn't show. By the way, F1 and F3 were found stable during 3 months at 8 °C, 25 °C. Results of characterization studies were shown in Table 2.

Table 2: The Characterization of Formulations

E	MA	C	S 8°C- 25°C/ 40°C	pH
o/w	Homogeneous, compact, white	177 µS	>3m / 1m	4.82
w/o	Homogeneous, white	-	<1m / 1m	-
w/o	Homogeneous, compact, white	374 µS	>3m / 1m	7.23
w/o/w	Homogeneous, white	-	<1m / 1m	-

* E: Emulsions, MA: Macroscopic Aspect, C: Conductivity, S: Stability

CONCLUSION

Utilization of the ostrich skins pre-fleshing waste which is one of the solid wastes of tanning industry is an eco-friendly approach. It is also important to decrease the cosmetic production cost by using less expensive substrates. In conclusion, the new suitable emulsion formulations could be suggested using ostrich oil as oil phase. Antienflamatuar activity, moisturizing effect and skin irritation of these formulations will be investigated in the future.

References

1. http://www.eshni.gov.uk/ostrichandenua5leaflet.pdf
2. http://www.ostrich-oil.com/ostrich-oil/default.htm
3. Sergio Escobar, Processing of the Fat in Commercial Oil of Ostrich. World Ostrich Congress-Chile 2003.
4. Ö. Özer, E. Balo lu, G. Ertan, V. Muguet, Y. Yazan, The effect of type and concentration of the lipophilic surfactant on the stability and release kinetics of the W/O/W multiple emulsions, International Journal of Cosmetics Science, 22, 459-70, 2000.

PO143

Natural surfactants as prospective pharmaceutical excipients for topical vehicles containing different oils: rheological characterization

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INTRODUCTION

Results obtained from the previous study [1] pointed to the possible use of a natural surfactant of alkylpolyglucoside type (cetearyl glucoside mixed with cetearyl alcohol) as an effective pharmaceutical excipient for dermatological and cosmetic formulations. The specific aim of this study was to investigate how the type of oil phases, i.e. the polarity degree, influences the viscoelastic/rheological properties and the structure of emulsion vehicles based on three different surfactants of alkylpolyglucoside type. In the first stage of this study nine test samples with fixed surfactant/water ratio (1:10.43) and 20% (w/w) of oil phase, were prepared and labelled as follows: F1(MG)-F3(MG), with medium chain triglycerides (MG), F1(OL)-F3(OL), with olive oil (OL) and F1(LP)-F3(LP), with light liquid paraffin (LP). The series of cream samples F1-F3 contained three different surfactants: cetearyl glucoside and cetearyl alcohol (M68), coco-glucoside mixed with cetearyl alcohol (M82) and arachidyl glucoside and arachidyl behenyl alcohol (M202), respectively. Since the concentration of 7% (w/w) arachidyl glucoside mixed emulsifier and 20% (w/w) of medium chain triglycerides did not provide semisolid pharmaceutical base for topical use, in the second part of this study three more samples containing 20% (w/w) of medium chain triglycerides (MG) and 10% (w/w) of alkylpolyglucoside surfactant were prepared and labelled as: F1(10MG) - F3(10MG).

EXPERIMENTAL METHODS

Rheological measurements

Rheological measurements were performed employing the rheometer (Rheolab MC 120, Paar Physica Stuttgart, Germany) coupled with the cone and plate measuring device, at 20 ± 0.1°C. The thickness of the sample in the middle of the sensor was 0.05 mm. During continual flow testing, controlled shear rate procedure was applied (shear rate from 0 to 200s^{-1} and back to zero). To identify possible presence of lamellar liquid or gel-crystalline domains, oscillatory measurements were carried out. Firstly, a viscoelastic region for all the samples was determined (amplitude sweep ramp), at constant frequency of 1 Hz. A frequency sweep ramp from 0.1–10 Hz was performed at constant strain (1%), which was within the previously marked linear viscoelastic region for all the samples.

Microscopic characterization

Samples were examined microscopically between crossed polarizers using the photomicroscope with λ plate (Leika DMR, Germany), magnification 400.

pH and conductivity measurements

pH values were measured by immersing the probe of the instrument (HI 8417, Hanna Instruments, USA) directly into the sample. Measurements of electrical conductivity were performed in the same course using conductometer CDM 230 (Radiometer, Denmark).

RESULTS AND DICSUSSION

Rheological measurements

All the samples exhibited the non-Newtonian „shear-thinning" pseudoplastic flow behaviour, the up-curve has not coincide with the down-curve, reaching slightly to moderately pronounced thixotropy (data not shown). Typical results of viscoelastic rheological tests a week after the preparation of test samples are given in Table 1 and Fig. 1. Considering the continual flow (η_{max}) and viscoelastic rheological test results (G' and G"), alkylpolyglucoside mixed emulsifier with the shortest alkyl chain (M82) has shown the best affinity for the most polar oil phase (OL). On the other hand, it appears that arachidyl glucoside mixed emulsifier, with the longest alkyl chain, developed the best lamellar gel phase with non-polar LP (the highest elastic modulus and satisfied tan value, Table 1), at least in the ratio used in this study. In both series of investigated samples cetearyl glucoside mixed emulsifier has exhibited

the most pronounced interaction with moderately polar MG. Variations in rheological profiles obtained for different oils and surfactants may suggest that potentially different mode of their packaging within the lamellar mesophase have taken place during structure formation. It is also known that such differences in the rheological properties may be influenced by water distribution mode within systems, reflecting on skin moisturization potential and drug release/penetration profiles.

Table 1: Oscillatory (at frequency of 1Hz) parameters of emulsion vehicles with emulsifier/water ratio 1:10.43

Sample	G'	G"	tanσ	η*
F2(LP)	273	402	1.47	7.74
F2(OL)	634	687	1.08	149
F2(MG)	311	347	1.12	7.42
F1(LP)	352	347	0.986	78.7
F1(OL)	932	839	0.901	200
F1(MG)	1000	736	0.734	163
F3(LP)	4730	1210	0.256	778
F3(OL)	1900	1340	0.708	370
F3(MG)	-	-	-	-

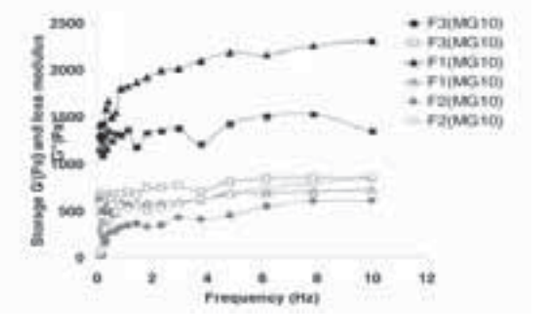

Figure 1: The storage G' (closed symbols) and loss (G") moduli (open symbols) of emulsion vehicles with emulsifier/oil ratio 1:2

Additionally, in all emulsion vehicles stabilized with cetearyl glucoside or arachidyl glucoside mixed emulsifiers the elastic moduli were higher than viscous ones (Table 1). According to some authors [2] such behaviour corresponds to the lamellar mesophase described as a weak gel. On the contrary, in the coco-glucoside based systems overlapping of loss and storage moduli at lower frequencies, tan σ >1 and the narrowest viscoelastic region were noticed, which may suggest an additional mesophase alongside the lamellar one.

Microscopic characterization

Polarized light micrographs of M68 and M202 samples with MG as oil phase (F1(10MG) and F3(10MG)) indicate the presence of lamellar gel phases within the continuous phase as well as "onion ring" structures (Fig. 2a and 2c), which were in depth described in pharmaceutical bases stabilized with traditional emulsifying waxes [3]. This complex microstructure is only partially observed in emulsion vehicles with M82 (F2(10MG)). However, it could be assumed that M82 stabilizes investigated samples creating lamellar gel phase and additional mesophase (Fig. 2b). The same phenomena have been seen in other investigated samples with this emulsifier.

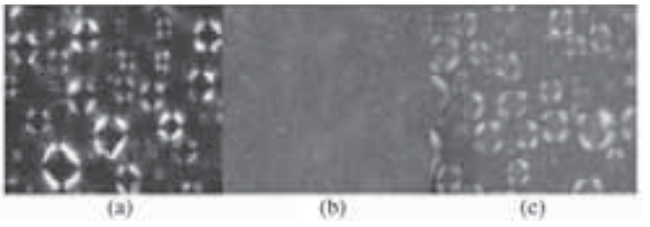

Figure 2: Polarization micrographs of investigated samples: (a) F1(10MG), (b) F2(10MG) and (c) F3(10MG)

pH and conductivity measurements

The pH values of all samples were within the range typical for the healthy human skin (4.0-6.5). Results of conductivity measurements show that cetearyl glucoside and arachydil glucoside based samples, independent on lipophilic excipients used, held higher portion of interlamellar water than coco-glucoside emulsifier. These findings are in line with better developed lamellar gel phase and more elastic nature of their colloidal structure.

CONCLUSION

The present study showed that structure of surfactant of alkylpolyglucoside type and oil phase polarity influence the colloidal structure of vehicles, particularly in terms of water distribution mode within the system and consequently their rheological performance.

References

1. S. Savic et al. Eur J Pharm Sci 30: 441-450 (2007).
2. O. Robles-Vásquez et al. J Colloid Interface Sci 160: 65-71 (1993).
3. G.M.Eccleston et al. Int J Pharm 203 (1-2): 127-139 (2000).

PO144

Rheological study and drug release of acyclovir hydrogels

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INTRODUCTION

Acyclovir, is a , one of the most commonly-used antiviral drugs, it is primarily used for the treatment of infections. Herpes simplex infections are all over the world, over 90 % of adult present signs of latent infection with HSV-1. In the case of labial herpes topical pharmaceutical forms have many advantages over the oral forms [4].

The aim of our study was to formulate and characterize in vitro acyclovir gels for topical application and to compare them, with generic, industrial medicines. Acyclovir containing topical application forms prepared on industrial scale are creams and ointments.

EXPERIMENTAL METHODS

Materials: Acyclovir (Avalon Pharma), Carbopol 940 (Noveon), Ultrez 21 (Noveon), methylcellulose (Fluka), triethanolamine (Fluka), distillated water , industrial creams: I, II and III.

Preparations of gels: methylcellulose gel was prepared using 5% of methylcellulose, Carbopol 940 and Ultrez 21 gels containing 1% of gel forming agent, and the dispersions were neutralized with triethanolamine. In these gels 5% acyclovir was suspended, because its pour water-solubility.

Rheological measurements were carried out with a RheoStress 1 HAAKE rheometer. A cone-plate measuring device was used in which the cone angle was 1º and the thickness of the sample was 0.048 mm in the middle of the cone. The measurements were performed at a constant temperature (25 °C ± 0.1 °C).

The linear viscoelastic range was determined in the first step by examining the complex modulus as the function of shear stress at a given frequency (1 Hz). Based on these experiments, the value of shear stress was set at 2.5 Pa during the dynamic test as this value was always within the linear viscoelastic range (LVR). Besides the oscillation tests, flow curves and viscosity curves of the different samples were also determined. Dates had been analyzed with Rheocalc for Windows 1.01 software. The determinations had been recorded for ten sequentially measurements (increasing and decreasing values of shear rate in the range 0,995 – 99,4 s^{-1} at a constant temperature.

Spreadability. About 1 g of gel was pressed between the surfaces of graduated glass plates (the upper plate had 54,1 g), on which weights of 50, 100, 200 and 500, 600 g were placed at intervals of 1 min. The diameters during each interval are given as the area (cm^2). The variations of the area as a function of weight were then analyzed as response factors [1,3].

In vitro release studies were carried out using Franz-type diffusion cells with a receptor compartment volume of 33.2 ml and an effective diffusion area of 3.14 cm^2. Standard cellophane membrane (Visking Tubing UK, size 18/32) was soaked in distilled water for 24 h before experiment. Distillated water was used as the receptor medium and 3 g of sample was placed on the donor side. The receptor was continuously stirred at 600 rpm and thermostated at 37 ± 0.5 °C with a circulating jacket. At certain time intervals, samples were withdrawn from the receiver compartment and replaced with an equal volume of fresh water. For each acyclovir containing sample, quantitative determinations were realized with a HPLC method, using the system: 1100 Agilent Technologies; Column: LiChrospher C18; 250 x 4 mm, 5 mm (Teknokroma), Pre-column C18; Mobile phase: 92% KH_2PO_4 25 mM pH 4,8 with H_3PO_4 85%, 8% acetonitrile; Flow rate: 0-3,49 min - 1 ml/min; 3,5-7,9 min – 1,5 ml/min; 8 min – 1 ml/min; Column temperature:30 °C; wavelength: 250 nm; injection volume: 10 µl; time 8 min [1,2].

The cumulative release amounts of acyclovir of tested formulations are plotted against of time.

RESULTS

Table 1 shows the critical stress and the elastic modulus values of the prepared hydrogels.

Table 1: Critical stress of acyclovir hydrogels – oscillatory rheometry

Acyclovir hydrogel-base types	Critical stress (Pa)	G` at LVR
Methylcellulose 5 %	5,4	3,7
Carbopol 940 1%	18,5	8,3
Ultrez 10 1%	19,0	10,2

Extensometric area (curves) are presented in figure 1.

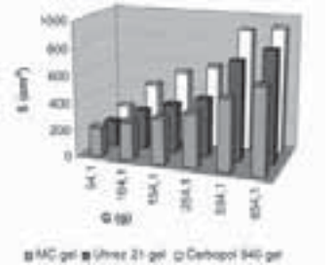

Figure 1: Extensometric curves of the prepared gels.

The cumulative acyclovir release from the prepared hydrogels and industrial creams is presented in figure 2.

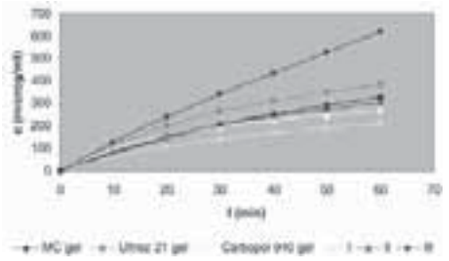

Figure 2: Comparative release of acyclovir from the prepared hydrogels and industrial creams

CONCLUSION

The elastic modulus (*G'*) was used as the response variable during preliminary experimentation as it was the most sensitive parameter in viscoelastic measurements. The results indicate that the presence of acyclovir did not significantly influence the gel-structure.

At stresses below the critical stress, the sample behaves like a viscoelastic solid. At higher stresses, the material starts to flow as its yield value is exceeded. To maintain consistency, the critical stress values (Table 1) were obtained from the cross over point of the phase angle (δ) values with the *G'* curves. Different acyclovir hydrogels with varying viscosity values (data from vendor generated by conventional viscometry) exhibited comparable critical stress values when probed using oscillation rheometry.

Carbopol 940 based acyclovir gel shows pseudoplastic flow, the viscosity decreases with increasing shear rates.

Among the three studied gels methylcellulose and Ultrez 21 hydrogels present thixotropic behavior. The hysteresis loop area may be used as an index of the degree of structural breakdown.

All the hydrogels present adequate spreadability, but Carbopol 940 gel presents the highest value.

The drug release decreases in the following order: Ultrez 21 gel > Carbopol 940 gel > Methylcellulose gel.

Compared the drug release from the industrial creams with the studied hydrogels, the Ultrez 21 based gel shows similar dissolution profile.

The differences in drug release can be explained by the different ointment base: we prepared hydrogels and pharmaceutical industry creams (o/w emulsions).

In conclusion, this study suggests, that Carbopol 940 and Ultrez 21 acyclovir gels can be recommended for laboratory- and industrial- scale preparation.

References

1. Aulton M. E.: Pharmaceutics, 2th edition, 2002, Elsevier, London.
2. Bolieiu R., Gallant C., Silberstein N.: Determination of acyclovir in human plasma by high-performance liguid chromatography, J. Chromatogr. B, 1997, 693, 233-236.
3. Erős I., Thaleb A.: Rheological Studies of Creams. I. Rheological Functions and Structure of Creams, Acta Pharm. Hung., 1994, 64, 199-207.
4. *** European Pharmacopoeia, 6th Edition, 2007, Council of Europe, Strasbourg.

PO058

Effect of the multiple surfactant system on physical properties and *in vitro* release of mupirocin from the cream base

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INTRODUCTION

In order to produce a stable cream emulsion of desired consistency it is of high importance to choose the right emulsifying system. Usually, semisolid emulsions contain more than one emulsifier. Commonly used combination is of surfactants and fatty alcohols. The multiple surfactant system evaluated in this study includes the mixture of amphiphilic compounds, like fatty alcohols (Cetyl alcohol and Stearyl alcohol), with the non-ionic surfactant (Cetomacrogol 1000 -Polyoxyethylene alkyl ether). Such amphiphilic compounds, though relatively weak emulsifying agents, can provide increased emulsion stability and better control of consistency when used in combination [1].

The ratio of emulsifiers in formulation can affect, apart from the emulsion stability and viscosity, the drug release profile. Therefore, in order to achieve the desired properties of the formulation, amount of emulsifiers have to be carefully optimized.

Active substance in Mupirocin cream is Mupirocin calcium dihydrate suspended in cream base. Mupirocin is a topical antibiotic used for treatment of impetigo and other superficial primary and secondary skin infections [2].

The aim of the present study was to investigate the influence of the emulsifiers ratio in the cream formulation on the release profile of active substance, emulsion stability and its viscosity.

EXPERIMENTAL METHODS

• Sample preparation

Samples with the different ratios of the emulsifiers were prepared, see Table 1.

Table 1: Samples with different amounts of the emulsifiers

Sample	Cetomacrogol 1000 (% w/w)	Cetyl alcohol (% w/w)	Stearyl alcohol (% w/w)
S-1	6.0	2.0	5.0
S-2	6.0	5.0	2.0
S-3	6.0	2.5	2.5
S-4	3.0	2.5	2.5
S-5	4.0	3.0	3.0

• Viscosity measurement

The viscosity profiles were obtained by measuring viscosity using Brookfield Rheometer RVDV-III ULTRA with spindle LV-3C, at various shear rates in the range from 0.02-0.11 s^{-1} at 25°C.

• *In vitro* drug release

The release of mupirocin from the samples was performed using Franz diffusion cells. Mixture of USP acetate buffer pH 5.5 and ethanol in the ratio 50:50 was used as receptor media and thermostated at 32 °C. Regenerated Cellulose (RC) membranes with 0.2 μm pore size were used to separate the donor and receptor compartment. The test lasted for 6.5 hours and the samples were taken at defined time points. Data obtained were plotted as the amount released per unit membrane area (μg/cm^2) versus square root of time [3].

• Stability of emulsion

The samples were stored in transparent bottles in the thermostat at 40°C. Possible phase separation in samples was examined visually after 4 months.

RESULTS AND DISCUSSION

It is well known that viscosity can influence the physical stability of the cream. It can also affect product performance such as spreadability, appearance, dispensing product from a tube, bottle or jar [4]. Effect of emulsifiers ratio on Mupirocin cream viscosity was tested by applying their different ratios as it is defined in Table 1. Results are shown in Figure 1.

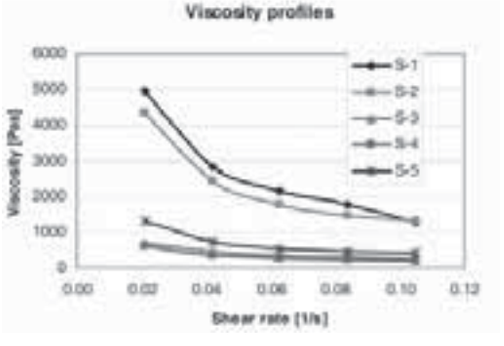

Figure 1: Comparison of viscosity profiles of samples S-1, S-2, S-3, S-4 and S-5.

Results (Figure 1.) indicate that samples with the different ratios of the emulsifiers differ in their viscosity profiles. Viscosity increases with the increase of the total amount of Cetyl alcohol and Stearyl alcohol in formulation, which is expected since they also act as stiffening agents.

In vitro release test was performed to evaluate how different ratios of emulsifiers affect the release rate. Results are presented in Figure 2.

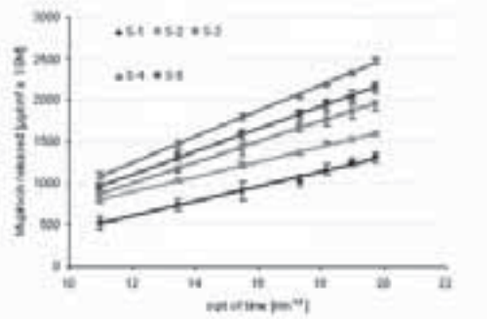

Figure 2: Comparison of In vitro drug release profiles from different formulations.

Generally results show (Figure 2.) that by increasing the total amount of emulsifiers in formulation the release of mupirocin from cream base is decreased. Based on these results, it was assumed that a lower concentration of emulsifiers should be applied to get an appropriate release rate of mupirocin from cream base.

On the other hand the emulsifiers in emulsion are employed to stabilize the system and avoid the possibility of creaming, droplet flocculation or coalescence [4]. Therefore its concentration in the formulation should be optimized. The separation of the emulsion can possibly lead to the non homogeneous drug distribution as well as to variations in drug release. In order to evaluate the physical stability of prepared formulations, samples were stored at 40°C for 4 months.

Table 2: Observations of phase separation on samples stored at 40 °C for 4 months; + observed, - not observed

Trial formulation	Phase separation/ after 4 months at 40°C
S-1	-
S-2	-
S-3	-
S-4	+
S-5	+

The data presented in Table 2. clearly indicate that at the lower total concentration of emulsifiers in samples physical stability is not assured. Therefore higher concentration of emulsifiers (total concentration of 11-13% w/w) is needed to maintain physical stability of the emulsion and avoid phase separation.

CONCLUSION

According to the presented results, it can be concluded that the ratio of Cetyl alcohol, Stearyl alcohol and Cetomacrogol 1000 have great impact on viscosity, release rate of mupirocin from cream base, as well as

stability of the emulsion. In order to achieve good physical properties and desired release of mupirocin, the concentrations of mixed emulsifiers should carefully optimized considering all these three parameters.

References

1. L. H. Block, Pharmaceutical Emulsions and Microemulsions, in: H. A. Lieberman, M. M. Rieger and G. S. Banker: Pharmaceutical dosage forms: Disperse systems, Marcel Dekker Inc., New York, 1996, p. 55.
2. M. A. Parenti, S. M. Hatfield and J. J. Leyden: Mupirocin: A topical antibiotic with a unique structure and mechanism of action, Clinical Pharmacy, 1987, p. 761.
3. FDA guidance for industry: non sterile semisolid dosage forms – Scale-Up and Postapproval Changes; Chemistry, Manufacturing and Controls; *In vitro* Release Testing and In Vivo Bioequivalence Documentation SUPAC-SS, January 2007.
4. M. Ramchandani and R. Toddywala, Formulation of topical drug delivery systems, in: T. K. Ghosh, W. R. Pfister and S. I. Yum: Transdermal and topical drug delivery systems, Interpharm Press, Inc., 1997, p. 554-560.

PO146

Characterisation of labrasol® / solubilisant gamma® based microemulsions as potential vehicles for transdermal delivery of ibuprofen

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INTRODUCTION

Microemulsions are thermodynamically stable and optically isotropic transparent colloidal systems currently of interest to the pharmaceutical scientist as promising drug delivery vehicles due to their long term stability, ease of preparation, low toxicity and irritancy, considerable capacity for solubilization of a variety of drug molecules and great potential in bioavailability improvement (1, 2). Microemulsion formulations have been shown to be superior for both transdermal and dermal delivery, particularly of lipophilic compounds, compared to conventional vehicles. Efficacy of lipophilic drugs is often hindered due to their poor aqueous solubility which limits their efficacy and bioavailability (3, 4). Ibuprofen (IB) is a non-steroidal anti-inflammatory drug, widely used in topical preparations in order to reduce side effects and avoid first-pass metabolism. Over the physiological pH range of the dermal tissues (4.0-7.4), IB displays low an pH-dependent both, solubility in water and skin permeability (5). There is a growing interest during the past decade for development of biocompatible microemulsions based on nonionic surfactant Labrasol® (PEG-8 caprylic/capric glycerides) as vehicles for cutaneous drug delivery (4). Interest in using nonionic tensides both, as a surfactant and as a cosurfactant (so-called non-alcohol cosurfactants), is increasing due to high stability, low toxicity, low irritancy and biodegradability of many nonionic surfactants (1-4). The purpose of the current study was to formulate microemulsion vehicles based on Labrasol® and new commercially available surfactant mixtures of polyoxyethylene types (Solubilisant gamma® 2421 and Solubilisant gamma® 2429) as cosurfactants, as well as to investigate and compare the effect of the cosurfactants and the oil phase on *in vitro* drug release parameters of IB from the microemulsion vehicles.

EXPERIMENTAL METHODS

Materials

Surfactant, Labrasol® (PEG-8 caprylic/capric glycerides) (LAS) and cosurfactants, Solubilisant gamma® 2421 (Octoxynol-12/Polysorbate 20) (SG2421) and Solubilisant gamma® 2429 (Octoxynol-12/Polysorbate 20/PEG-40 Hydrogenated castor oil) (SG2429), were kindly donated by Gattefosse, France. Oils, isopropyl myristate (Crodamol® IPM) (IPM) and medium chain triglycerydes (Crodamol® GTCC) (GTCC), were purchased from Croda Chemicals Europe, England. Water was double-distilled with conductance < 3 μS/cm.

The microemulsion vehicles were formed spontaneously at room temperature by admixing appropriate quantities of surfactant, cosurfactant, oil, and water at surfactant/cosurfactant mass ratio 1:1, oil to surfactant/cosurfactant mixture mass ratio 1:9 and water content of 45 % *w/w*. Additionally, IB was dissolved into preweight vehicles at a concentration of 5 % *w/w*. Samples were maintained at room temperature for a 48 h, before their characterization.

Polarized light microscopy

In order to verify the isotropic nature of microemulsions, samples were examined using cross-polarized light microscopy (Leitz Wetzlar 307-083.103 514652, Germany).

pH

The pH values of the microemulsions were measured by a pH meter (model HI 8417, Hanna Instruments Inc., USA), at 20 ± 1 ºC

Conductivity measurements

Electrical conductivity (σ) of the samples was measured using a conductometer CDM 230 (Radiometer, Denmark), at room temperature and the frequency of 94 Hz.

Rheological measurements

Apparent viscosity (η`) 20 ± 0,1 °C, was measured using a rotational rheometer (Rheolab MC120, Paar Physica, Germany) coupled with cup and bob measuring device Z3 DIN.

Droplet Size

The average droplet size (Z-ave) and polydispersity index (PdI) of samples were determined by photon correlation spectroscopy (Nano ZS90, Malvern Instruments, U.K.) using a He–Ne laser at 633 nm and. The measurements were performed at 90º scattering angle and at temperature of 20 °C.

In vitro drug release study

In vitro release profile of IB through regenerated cellulose membrane was determined using a rotating paddle apparatus (Erweka DT70, Germany) (the rotating paddle speed was 100 rpm) modified by addition of dissolution cell (VanKel Industries, Inc., USA), at 32°C. Drug concentration in the receptor medium (pH 7.4 phosphate buffer) was determined spectrophotometrically at λ=220 nm.

RESULTS AND DISCUSSION

In the present study, the obtained values of pH, η` and σ (Tab. 1) indicate a successful formulation of pharmaceutically acceptable, low viscous, oil-in-water microemulsions, which isotropic nature was confirmed by polarized light microscopy.

The stability of microemulsion vehicles was not compromised by dissolving of IB, in spite of water-continuous nature of the vehicles and poor water solubility of the drug. Furthermore, small changes in pH and s values can be ascribed to the predominant partition of IB into the oil/surfactant phase of the microemulsions. Significant decrease of apparent viscosity values in LAS/SG2421 based microemulsions, pointed out the possibility of the stronger interactions of IB with SG2421 cosurfactant, while in the presence of SG2429 the viscosity remains unchanged by the drug (Tab. 1).

Table 1: The values of pH, η` and σ for microemulsions vehicles and microemulsions containing IB (marked in the superscript)

Sample*	SG2421/ IPM	SG2421/ GTCC	SG2429/ IPM	SG2429 GTCC
pH	5.85	5.72	6.33	6.21
pH^{IB}	4.70	4.72	5.16	5.21
σ (μS/cm)	42.2	40.8	78.6	75.6
σ^{IB} (μS/cm)	33.6	34.8	57.00	59.4
η' (mPas)	221.45	232.86	323.60	404.69
η'^{IB} (mPas)	127.38	144.95	323.60	404.70

* the samples are marked by the cosurfactant and the oil phase used for their preparation

The results of the droplet characterization of the microemulsions containing IB (Tab. 2, Fig. 2, Fig. 3) showed the presence of small droplets and very low polydispersity. Also, it was observed smaller droplet diameter for the microemulsions with medium chain triglycerydes (GTCC) compared to fatty acid ester (IPM) in case of both cosurfactants.

Table 2: Z-ave and PdI of IB loaded microemulsions

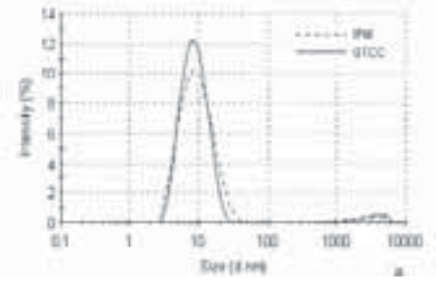
Sample	SG2421/ IPM	SG2421/ GTCC	SG2429 IPM	SG2429 GTCC
Z-ave (nm) ± S.D.	8.29 ± 0.01	8.07 ± 0.03	8.40 ± 0.03	5.33 ± 0.04
PdI ± S.D.	0.236±0.004	0.212±0.001	0.224±0.005	0.254±0.002

This can be explained by the well documented (6) observation that the small volume oils may penetrate the hydrophobic chain region of the surfactant monolayer behaving as a 'cosurfactant' and influencing the droplet size.

The release profiles of IB from the investigated microemulsions (Fig. 2) correlate well with *Higuchi* model. Diffusion rate constant (k) and cumulative amount of IB released after 6 h (Q) (Tab. 3) were afected by both, the cosurfactant and the oil. These results correlated well with the assumptions of possible IB/SG2421 interactions with decreasing influence on Q and k values as well as of IPM/LAS/cosurfactant interactions which enhance IB release parameters.

Table 3: In vitro IB release parameters

Sample	SG2421/ IPM	SG2421/ GTCC	SG2429/ IPM	SG2429/ GTCC
Q (%, 6 h)	14.47	11.70	15.33	12.45
k ($mgh^{1/2}$)	7.00	5.24	7.20	5.46



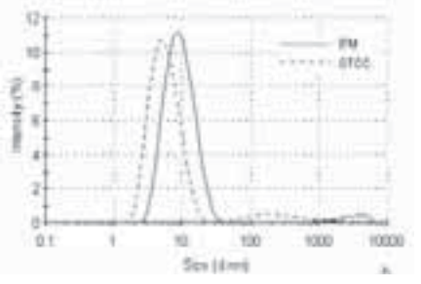


Figure 1: The influence of oil phase on size distribution by intensity for: a) LAS/SG2421 based microemulsions; b) LAS/SG2429

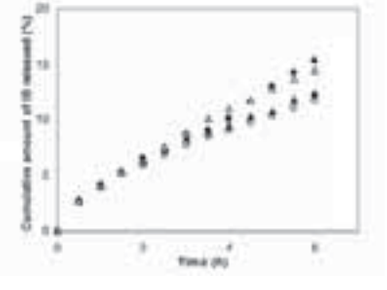


Figure 2: In vitro IB release profiles: SG2421/IPM (△), SG2421/GTCC (o), SG2429/IPM (●), SG2429/GTCC (▲)

CONCLUSION

The obtained data suggest that Labrasol®/ Solubilisant gamma® based microemulsions are promissing vehicles for poorly water soluble drugs. The results of *in vitro* release of model drug (IB) revealed the superior cosurfactant (SG2429) and oil phase (IPM) regarding the duffusion od the drug through the cellulose membrane.

References

1. Bagwe et al., Crit. Rev. Ther. Drug Carrier Syst., 18 (2001) 77-140.
2. Gupta and Moulik, J. Pharm. Sci., 97 (2008) 22-45.
3. Heuschkel et al., J. Pharm. Sci., 97 (2008) 603 – 631.
4. Kreilgaard, Adv. Drug. Del. Rev., 54 (2002) S77–S98.
5. Brain, K.R., James, V., Walters, K.A. (Eds.), Prediction of Percutaneous Penetration, STS Publishing, Cardiff, 1993.
6. Warisnoicharoen et al., Int. J. Pharm., 198 (2000) 7-27.

PO147

Development of *in vitro* drug release method for semisolid dosage forms: case study adapalene cream

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INTRODUCTION

During development of semisolid dosage form there is a need to optimize the delivery of drug into and through skin to provide maximum therapeutic effect. Therefore a development of *in vitro* release method is prerequisite for evaluation of delivery system [1].

In vitro release method measures diffusion described by Higuchi model. The quantity of drug released is proportional to total concentration of drug in delivery system, a concentration of drug solubilised in matrix, a diffusion coefficient and time.

$Q = \sqrt{2ADC_s t}$

In vitro release test is often used during development for delivery system optimization and control of product quality and performance over a time.

During method development a several parameters have to be carefully defined like temperature, membrane, receptor medium and sample collection times.

The aim of this study was development of *in vitro* release method for Adapalene from Adapalene cream.

EXPERIMENTAL METHODS

Apparatus

In general a system for release testing consists of donor compartment where sample is placed and of receptor compartment which holds the receptor medium. Two mostly used apparatus are Enhancer cell and Franz diffusion cells.

Franz diffusion cells with automatic sampling was apparatus used for *in vitro* release method development for of Adapalene from cream

Membranes

The membrane provides physical support to samples. It should be chemically inert toward the active substance and should not be rate-limiting in release process. It is important that the membranes are conditioned in receptor medium prior to an experiment.

Several artificial membranes were evaluated: cellulose acetate with 0.8 μm, polytetra-fluoroethylene (PTFE), 0.45 μm and regenerated cellulose membranes with 0.2 μm.

Receptor medium

Solubility of the active substance in the receptor media is the most important factor for in vitro release testing. The receptor collects the drug without limiting its transport, and has to exhibit good solvent properties to maintain sink conditions.

Since Adapalene is practically insoluble in water and ethanol, isopropylmiristate (IPM) was added to their mixtures to increase solubility in receptor medium.

Three mixtures of IPM, ethanol and water presented in Table 1, were used as receptor media for *in vitro* release testing of two samples of Adapalene cream differing in excipient composition.

Table 1: Receptor media composition

Medium 1	IPM:Ethanol:Water 5:90:5
Medium 2	IPM:Ethanol:Water 5:85:10
Medium 3	IPM:Ethanol:Water 5:80:15

Temperature

Receptor temperature is generally set to 32 °C which is approximate skin temperature (1]. Therefore the same temperature was applied in this method.

Stirrer speed

Through the test receptor medium is constantly agitated with a magnetic stirrer. Stirrer speed should not interfere with diffusion surface area, and therefore it was set at 200 RPM in all tests during method development.

Sampling points
Collection of the sample should be carried out at specified time intervals. At least five sampling points should be provided. In vitro release testing for Adapalene cream lasted 6.5 hours and samples were withdrawn at defined time points.

Assay determination
Reliable, reproducible and sensitive assay method is crucial for determination of low drug concentrations in receptor medium. HPLC technique is commonly used for such method.
The analysis of Adapalene was performed on Agilent HPLC apparatus. The chromatographic conditions were as follows: reversed phase C-18 column, mobile phase acetonitrile: terahydrofurane: water: trifluoroacetic acid (42:32:26:0.02), flow rate 1ml/min, UV detection 235 nm.

Data analysis
Data are presented as the amount released per unit area (μg/cm^2) versus square root of time (min$^{1/2}$). Slope of the regression line presents the release rate of active substance.

Slopes obtained for six cells in Adapalene testing have been calculated and statistical analysis of release rates was evaluated according to current FDA Guideline [2].

RESULTS AND DISCUSSION
During method development different parameters were optimized in order to obtain final *in vitro* release method which is able to detect relevant differences among the formulations.
Franz diffusion cells apparatus was used. Temperature of receptor media was set to 32°C, and membrane regenerated cellulose with 0.2 μm was determined as the most suitable. It showed compatibility with Adapalene and no back-diffusion while PTFE membranes showed incompatibility and cellulose acetate membranes with 0.8 μm showed back-diffusion.

Results presented in Figure 1. showed that by increasing water content in receptor medium a release rate of Adapalene decreases. Statistical analysis of 90% confidence interval was performed and release rates were compared in each medium.

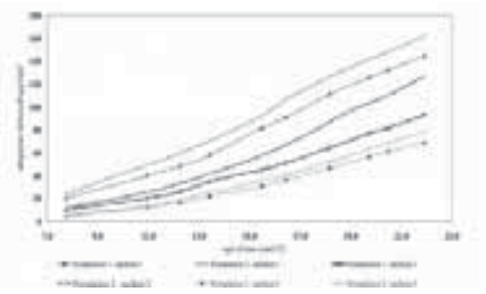

Figure 1: In vitro drug release profiles of two Adapalene formulations in different receptor media.

Differences between release rates were obtained in medium 2 and 3. However, due to incompatibility of IPM and water in ratio 5:15 in medium 3 which resulted in precipitation and phase separation, this medium was excluded from further testing. Medium 2 containing IPM: ethanol: water ratio 5:85:10 was defined in the method as suitable one. Duration of the test for 6.5 h was proven as suitable with seven sampling points.

CONCLUSION
Method development for *in vitro* drug release from semisolid dosage form was shown on the example of Adapalene cream. Method parameters were defined based on the release behavior of Adapalene from cream formulations. All tests were conducted on Franz diffusion cells. Results showed that during method development a special attention should be put on membrane selection as well as the receptor medium composition.

References
1. Zatz J.L., Segers J.D., Techniques for measuring *in vitro* release from semisolids, Dissolution Technologies, February 1998, p. 3-17.
2. FDA Guidance for industry: non sterile semisolid dosage forms – Scale-Up and Postapproval Changes; In vitro Release Testing and In Vivo Bioequivalence Documentation SUPAC-SS, January 2007.

PO148

In vitro case study: investigation of o/w cream containing sea buckthorn oil on skin moisture

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ABSTRACT
Skin moisture is influenced by presence of different emollients used in topical o/w emulsions. Since skin inflammatory processes cause increased trans-epidermal water loss, dermatological preparations which increase skin moisture content after its application show enhanced dermatological effects (1). The aim of this paper was to determine and com-

pare moisturizing potential of oleosom type o/w emulsion containing an emollient of natural origin (40% of fruit fatty sea buckthorn oil) and the same oleosom type o/w emulsion having the same amount of a synthetic emollient (isopropyl myristate) on the healthy skin of 12 volunteers.

INTRODUCTION

New types of emulsions commonly called "oleosoms" provide prolonged skin hydration effect due to the presence of water entrapped between the liquid crystal layers (2). These emulsions have dispersed drops of oil phase surrounded by the thick multi-layer of liquid crystalline lamellar surfactant, which protects them from coalescence by steric or electric repulsion. The thick liquid crystalline layer consists of surfactants with both low and high HLB values, such as ethoxylated stearyl alcohols and propoxylated stearyl alcohols and some stearyl alcohols.

Currently used emollients belong to the wide range of chemicaly different compounds: paraffinum liquidum, mineral or natural oils, waxes, wool fat, fatty acids, fatty alcohols, esters of fatty acids (isopropil palmitate or isopropyl myristate) or esters of polyhidroxyl alcohols (3).

Sea buckthorn fatty oil derives from sea buckthorn fruits. It contains polyunsaturated fatty acids, vit. E, β-caroten and carotenoids (4). Almost 80% of total fatty acids content are unsaturated fatty acids (oleic, linoleic, palmitoleic and linolenic). High content of two essential fatty acids, linoleic and γ-linolenic acids is caracteristic, 30–40% and 20–35%, respectively. The content of vitamin E has been found to be (64,4 - 481mg/100g fruits). Bright orange color of oil is due to present carotenoids. Fatty oil of sea buckthorn showed ameliorating effects on inflamed, damaged or dry skin providing dermo-regenerative, protective and anti-microbial effects (4).

EXPERIMENTAL METHODS AND MATERIALS

Samples of creams (o/w emulsions) C_1 and C_2 were prepared. Sample C_1 contained 40% of sea buckthorn oil (producer Hofigal S.A., Romania) – while sample C_2 contained the same amount of isopropyl myristate (Oleon, Belgium). For emulsion preparation we used the following materials: Arlamol® E (PPG-15 stearyl ether), Stearyl alcohol-(Lanette 18)-Cognis, Germany, Cetyl Alcohol-(Lanette 16)-Cognis, Germany, 5%-aliquots of the emulsifiers Brij® 72 (Steareth-2) and Brij® 721 (Steareth-21), Propylene glycol and purified water (Ph Jug V). The procedure used was advised by the producer: components of the oil phase and the emulsifing agent had been meleted on the water bath at temperateure of 75°C, while water phase was heated to 75-80°C. Phases were merged at 75-80°C with continuous mixing of 800 rpm and homogenization of 2000 rpm during 2 min forming oleosom type emulsions.

We examined and compared effects of samples C_1 and C_2 on the skin moisture and skin pH at 12 healthy women (average age 45.5). The samples were applied twice daily for 28 days after which there was a pause for one week during which there was no application. The last measurement was done on 35th day. The volunteers were aware that 3 days before the application of the examined preparation they should not apply any of dermatological or cosmetic products on the place of the application (the inner under-elbow surface of the skin). 20 min before the testing started, the volunteers have been placed in the room with constant conditions (temperature 22 ± 1°C, humidity 55 ± 5%). The measurements of the skin moisture were done by CorneometerCM 820 (Courage+Khazaka Elektronic, Germany) while skin pH by Skin-pH-meter PH900 (Courage+Khazaka Elektronic, Germany). The samples were applied in dose of 2 mg/cm^2 on the specified region (9 cm^2) of the insides of their forearms once a day, in the morning. The measurements of the skin moisture and skin pH were done before application of the samples C_1 and C_2 as well as on days 1, 3, 5, 7, 14, 21, 28 and 35.

Statistics: The Student's t-test ($p<0.05$) and ANOVA were used to evaluate the statistical significance of the measured differences for both samples.

RESULTS AND DISCUSSION

There were neither allergic reactions nor any other side effects detected during the testing. Once daily application of the oleosom type creams containing 40% of seabuckthorn fatty oil or isopropyl myristate did not significantly changed pH of the skin of healthy volunteers (Figure 1).

A statistically significant increase was obtained in relative skin moisture contents between the baseline value and those obtained after application of samples C_1 and C_2.

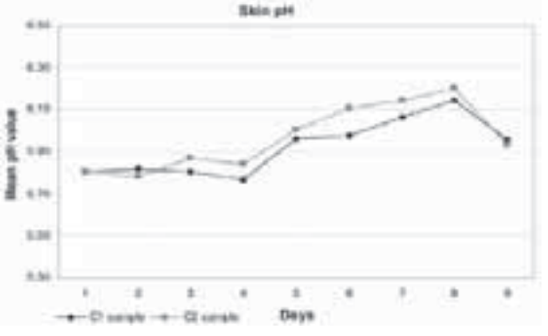

Figure 1: The mean skin pH value during the examined period.

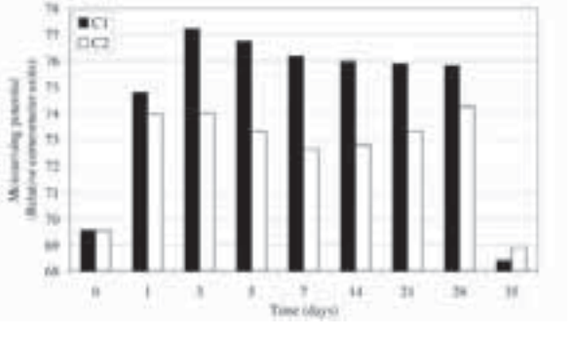

Figure 2: The change of skin moisture in time

The registered increase of skin hydration after twice daily application of the creams containing seabuckthorn fatty oil or isopropyl myristate confirms positive moisturizing effect of the emollients on the skin (Figure 2). There were significant changes of skin moisture for C_1 and C_2 samples compared to the base line values, registered on the day one after the application of sea buckthorn sample (C_1) which continued until the day

28 inclusive, while the change for the sample containing isopropyl myristate (C_2) was registered only after the day 1 and 3 after the aplication. The highest value for skin moisture obtained by C_1 sample was on the day 3 (increase of 10,31%). Statistically significant difference of moisturizing potential of samples C_1 and C_2 started on the 3 and continued until the end of examination.

A pause of one week influenced the skin moisture in the following way – sea buckthorn sample had a drop of 1,64% of base line value while isopropyl myristate sample a slightly lower drop (0,92%).

Both oleosom creams covered the skin surface lowering moisture evaporation which led to the increase of the skin hydration. The increase of skin moisture during 28 days of application of creams indicates that the moisturizing effect achieved is probably due to simultaneous presence of liquid crystals in the oleosom structure of creams and used emollients which both provided prolonged hydration of the skin. The enhanced moisturizing effect on the skin of the creams containing natural emollient (sea buckthorn fatty oil) we suppose that is due to higher affinity of numerous natural occurring constituents of sea buckthorn oil for epidermal skin cells then the affinity of a synthetic emollient such as isopropyl myristate.

CONCLUSION

Evaluation of twice-a-day application of the oleosom type o/w creams containing a natural or synthetic emollient (40% of seabuckthorn fatty oil and isopropyl myristate) showed significant increase of skin hydration. We concluded that a natural emollient possesses higher skin moisturizing potential with longer moisturizing effects then the synthetic one. Both formulations did not influence pH of the skin.

References

1. De Polo K.: Cosmetic emulsions, In: De Polo K., A short textbook of cosmetology, Verlag fur chemische industrie, H. Ziolkowsky GmbH, Augsburg, 1998.
2. McCallion R., Po ALW. Modelling transepidermal water loss under steady-state and non-steady state relative humidities. Int. J. Pharm. 1994; 105:103-112.
3. Kameshwari V. and Mistry D.: Sensory properties of emollients, Cosm. Toil. 1999; 114 (1): 45-51.
4. Yang B. and Kallio H., Comopsition and physiological effects of seabuckthorn (*Hippophaë*) lipids, Trends in Food Science & Technology, 2002; 13: 160-167.

PO149

Topical delivery of vitamins C and E from lipophilic microemulsions

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INTRODUCTION

Supporting skin endogenous antioxidant system is a popular strategy for skin protection from excessive exposure to free radicals. As the balance between skin antioxidants is very important, a combined therapy with a hydrophilic (vitamin C) and a lipophilic (vitamin E) antioxidant is desirable. Although many cosmeceuticals contain vitamin C and/or E, only few are effective after topical application, firstly because of poor (photo)stability of both vitamins and secondly because vitamins and their derivatives are not well absorbed or metabolized by the skin [1]. The specific structure of microemulsion (ME) allows the incorporation of lipophilic and hydrophilic antioxidant in the same system. ME are clear, thermodynamically stable dispersions of water and oil, stabilized by an interfacial film of surfactant molecules [2].

The purpose of this work was to evaluate the influence of non-thickened and thickened lipophilic (w/o) ME on release profiles and *in vitro* skin bioavailability of vitamins C and E.

MATERIALS AND METHODS

Vitamins C and E (purchased from Sigma Aldrich) were incorporated in different ME in 0,4% and 1% concentration respectively. w/o ME consisted of 30% of surfactant mixture (Imwitor 308:Tween40=1:1),10% water and 60% isopropyl myristate as oily phase. It was thickened by adding either 10% of colloidal silica (Degussa, Aerosil 200) or 10% of white wax (Pharmachem, Slovenia).

Release studies were performed using Franz cells and cellulose acetate membrane under sink conditions.

In vitro permeation studies were performed on pig ear skin. The amounts of vitamins accumulated in epidermis and dermis and passed into receptor solution were determined using Franz diffusion cells and infinite dosing after 6 hours of contact. All samples were analysed by HPLC.

Accumulation of colloidal silica inside *stratum corneum* was investigated by SEM after tape stripping.

RESULTS AND DISCUSSION

• Release studies

Release profiles of vitamins C and E are shown on fig. 1a&b.

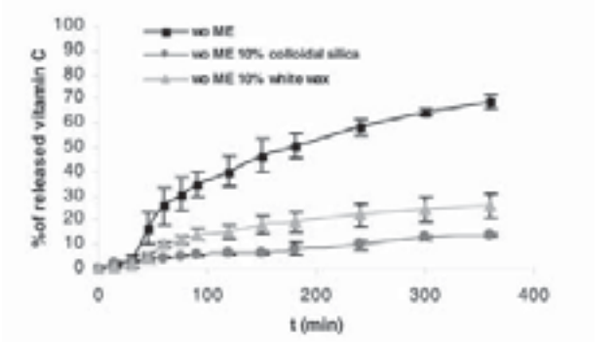

Figure 1a: Release profiles of vitamin C from different ME.

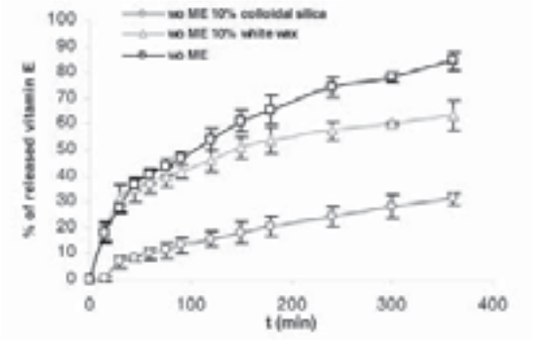

Figure 1b: Release profiles of vitamin E from different ME.

In all samples total amount of released vitamin was higher for vitamin E than for vitamin C. For vitamin C delayed release was observed which can be attributed to its partition to inner phase of ME whereas vitamin E was predominately located in outer phase. ME thickened with colloidal silica had higher viscosity (≈3900mPas) than ME thickened with white wax (≈1100mPas) which explains higher release rate of both vitamins from the latter.

• *In vitro* permeation studies

All ME were able to deliver vitamins C and E to the skin. Skin concentrations of vitamin E were approximately 100x times higher than of vitamin C (fig.2a &b) which indicates that *stratum corneum* is an efficient barrier for hydrophilic vitamin.

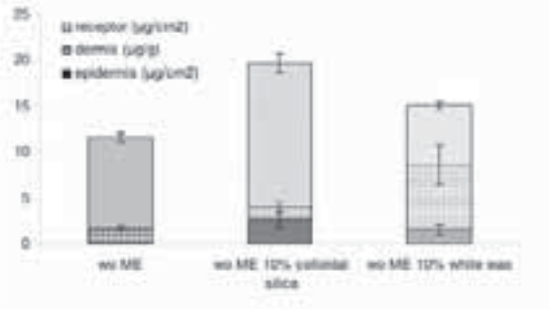

Figure 2a: The amounts of vitamin C accumulated in epidermis, dermis and receptor after 6 hours of contact.

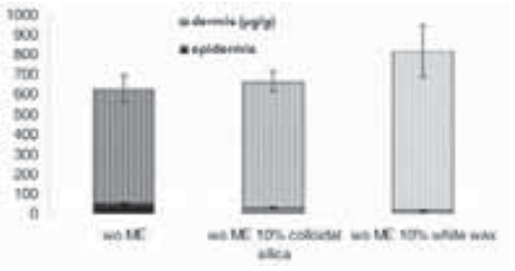

Figure 2b: The amounts of vitamin E accumulated in epidermis and dermis after 6 hours of contact.

Vitamin E, being lipophilic, easily permeated in dermis, where it bound to the tissue [3]. Its partitioning to receptor fluid was not favoured. On the other hand vitamin C had little affinity to dermis and permeated in considerable amounts into receptor. The addition of thickener increased skin retention of both vitamins. Colloidal silica increased accumulation of vitamin C in epidermis and also facilitated its passage into receptor. ME thickened with white wax delivered the highest amounts of vitamins in dermis.

• SEM picture

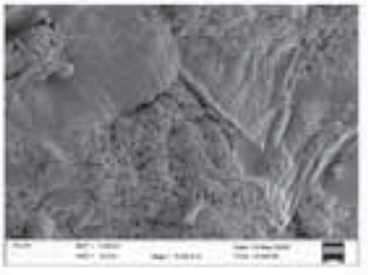

Figure 3: SEM picture of 2nd tape strip of pig ear skin treated with w/o ME thickened with 10% colloidal silica.

Colloidal silica accumulated to considerable amount in the first layers of *stratum corneum* (fig.3) but was not found in deeper layers. We assume that it disturbed the organisation of outer layers of *stratum corneum* and facilitated passage of hydrophilic vitamin across the skin (fig. 2a).

CONCLUSION

Results of release studies show that the release profiles of vitamins from ME are mostly influenced by vitamin location in vehicle and viscosity of formulation. Permeation studies confirmed tested ME as suitable carriers for simultaneous delivery of hydrophilic and lipophilic vitamin in the skin. No simple correlation between vitamin release and permeation could be found, mainly due to the fact that formulation can apart form vitamin release change also skin properties that were confirmed by SEM pictures.

References

1. Lupo, M. P., Clin Dermatol, 2001. 19(4): p. 467-73.
2. KOgan, A. and N. Garti, Adv in Colloid and Interface Sci,. 2006. 123-126: p. 369-385.
3. Lee, A. R. and K. Tojo, Chem Pharm Bull (Tokyo), 2001. 49(6): p. 659-63.

PO150

Computer-aided critical factor screening for ibuprofen release in Franz cells

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INTRODUCTION

The drug release studies using Franz cells (FC) involves formulation- and FC-related factors. Previous studies have been usually executed using one factor at a time (OFAT) approach[1]. OFAT does not reveal the factor interactions, if any and unable to capture the real cause and effect relationships. In contrast, computer-aided design of experiment (DoE) approach reveals critical factors, their interactions and suggests the best combinations of the factors for optimized outputs with lesser number of experiments[2,3]. Thus, OFAT surrenders in favour of DoE. This study aims to explore the critical FC-related factors for ibuprofen release using DoE approach.

EXPERIMENTAL METHODS

The factors included in the experimental design for optimization study were derived from preliminary pilot experimentation and literature. The six factors studied at two levels and included type of membrane (cellulose acetate, A and visking, B), time for membrane washing with 0.1 M NaOH (0.5 hr and 24 hr), sample frequency (frequent and infrequent), sampling site (bottom and centre of receiver), dimension of stirring bar (12.48 4.6 mm and 12.50 3.6mm) and pre-sampling mixing (no and yes). The response variables analyzed included cumulative release of 5% ibuprofen gel at 30, 60, 90, 120, 180, 240, 300, 360 and 420 min and the % coefficient variation (CV) at the corresponding release points. For screening of the critical factors, factorial design with minimum run enough for the resolution V implemented in Design Expert 7.1.5 (DX®7.1.5) was used.

RESULTS AND DISCUSSION

The study was completed with 22 experiments according to the design matrix generated by Design Expert 7.1.5. DX revealed membrane washing time and stir bar dimension as the most critical factors, as indicated by their $p<0.01$ in each case. For overall desirability of maximum drug release at all points and minimum %CV (less than 6%), no interactions attained level of significance. DX predicted, by a predictive model the following combinations of the factors to achieve the optimized output: membrane type; B, visking time; 24 hrs, sample frequency; frequent, sample site; centre, stirring with stir bar thickness; 3.6mm, premixing; no, provided the maximum ibuprofen release for all the time points and reasonably low %CV (Figures 1 & 2, respectively). Confirmatory experimental with predicted factors levels provided drug release and %CV within 10% of the predicted responses. Sampling site as critical factor is possibly due to the lower amount of drug at the receiver bottom than in centre despite stirring. The originally provided stir bar (12.50 ± 3.6 mm) showed favourable effect on release and CVs indicating the appropriateness of the mixing.

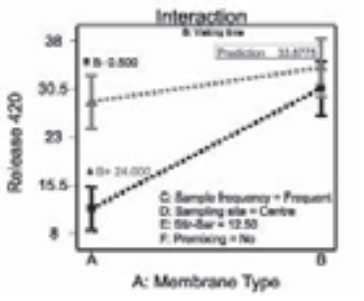

Figure 1: Interaction plot showing effect of membrane and visking time on drug release

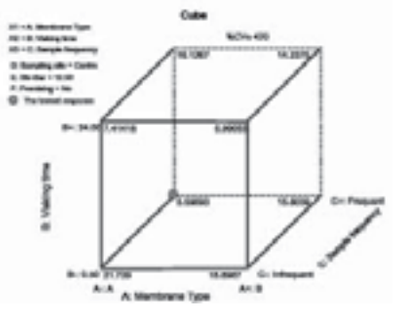

Figure 2: Cube plot showing the combined effect of membrane type, visking time and sample frequency on %CV

With OFAT for 6 factors, each at two levels requires number of experiments equal to 2^K (66), where k is the number of factors. With use of DoE, the same information was obtained by approximately 3 fold less number of experiments (22). With the knowledge of critical factors, the outcome can be optimized.

CONCLUSION

Design of experiment predicted critical factors and using the information, the release was optimised.

References

1. Fern, S. Ng. (2007). Investigation into the use of synthetic membranes for diffusion in Franz cells. *PhD Thesis*. University of Strathclyde, UK.
2. Lewis, G. A. Mathieu,D. & Tan-Luu, R. P. Ed. (1999). In: Pharmaceutical experimental design. Marcel Dekker, New York
3. Khuri, A. I. & Cornell, J. A. Ed. (1987). In: Response surfaces – designs and analyses. Marcel Dekker, New York.

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PO151

Characterization of different clopidogrel addition salts

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INTRODUCION

Salt formation is the key tool for properties variation of active pharmaceutical ingredients, and is well known and well documented. Different salts of the active compounds can be used to increase, or decrease solubility, to improve stability or toxicity to reduce hygroscopicity of the parent substance or increase bioavailability. Search in Cambridge Structural database (CSD) (2) showed high occurrence of active substances in the form of salts. It was found that chloride salts occurs in highest percentage, followed by bromide, nitrate (V), ammonium, sulfate (VI) etc salts. Many of this salts form different polymorphs and hydrates which are of great importance to pharmaceutical industry. Although identical in chemical structure polymorphs may differ in physical and chemical properties (1, 2)).

Clopidogrel (+)-methyl-(S)- -5-(o-chlorophenyl)6,7-hidroxythieno (3,2-a) pyridine-5 (4H) acetate is a potent anti platelet-aggregation drug. Hydrogensulphate salt is used in marketed product (3). Clopidogrel as a free base form is an colorless, oily substance with high viscosity which is relatively unstable and difficult to purify. Due to inappropriate physical properties and low water solubility of free base different acid addition salts were prepared. US patent 4,847,265 disclose four acid addition salts of clopidogrel hydrogen sulphate, hydrochloride, hydrobromide and taurocholate. Beside mentioned salts many others are known such as besylate, acetate, benzoate, maleate, citrate, mesylate (4).

Only limited data on characteristics and behavior of these salts can be found from the scientific literature. The solubility of drugs is important parameter for their bioavailability. The aim of our present work was thus to perform a comparative solubility study among different salts and some crystalline forms of clopidogrel.

Experimental

Materials

Clopidogrel hydrogen sulphate form 1 (HS F1), hydrogen sulphate form 2 (HS F2), clopidogrel hydrochloride (HCl) form 1, clopidogrel hydrobromode (HBr) form 1, clopidogrel besylate were supplied by Krka, d.d., Novo mesto.

METHODS

Thermal analysis (DSC) was performed on Perkin Elmer DSC 7 (dynamic N2 atmosphere, heating rate 10°C/min). Thermal effects were evaluated using Pyris software.

Solubility in aqueous solutions were determined in 0.1 M HCl solutions (pH 1), acetate buffer (pH 4,5), phosphate buffer (pH 6,8) and water at temperature 37°C. Excess of clopidogrel acidic salts (100 mg /ml) were added to 20 ml of buffer solutions and shaken 72 hours (incubated at 37°C). The concentration of clopidogrel in the filtrate (0.45 μm pore size filter) was determined by HPLC analysis.

Solubility in acetone. Excess of pure clopidogrel HS forms 1 and 2, 4 g was added to 50 ml of acetone and stirred for 20 min (incubated at 263 K, 273 K, 288 K, 303 K and 329 K). The concentration of clopidogrel in the filtrate (0.45 μm pore size filter) was determined by HPLC analysis. After sampling, polymorphic form of a solid residue was checked with IR to confirm, that no transition occurred during the dissolution process.

Dissolution profiles. Sample (75 mg) was added to 900 ml 0.1 M HCl at 37°C and mixed with paddle(s) (USP apparatus 2) at 50 rpm. The concentration of clopidogrel in the filtrate (0.45 μm pore size filter) was determined by HPLC analysis after 5, 10, and 15 min.

RESULTS AND DISCUSSION

Results of DSC analysis showed differences in melting parameters among different salts of clopidogrel (Table 1).

Table 1: Melting points and melting enthalpies of clopidogrel HS F1, HS F2, HCl, HBr, Besylate salt

Parameter	HS F1	HS F2	HCl	HBr	Besylate
melting point (°C)	185,5	181,67	143,3	114,5	136,67
melting enthalpy (J/mol)	31425	36363	51108	69353	44712

One of the practical properties of polymorphic substance is relative thermodynamic stability. From the melting parameters we can propose enatiotropic relationship between clopidogrel HS F1 and HS F2 and lower thermal stability of other salts of clopidogrel especially HBr.

Solubility of clopidogrel salts strongly depends on pH (Figure 1). Solubility of all salts is very high at pH 1. As expected with increasing pH the solubility is decreases. In 0.1 M HCl we observed lower solubility for HBr salt. This could be attributed to pKa differences between HBr and HCl acid. HBr is stronger acid than HCl.

Solubility's of clopidogrel HS F1 and HS F2 are similar in all pH range and a little higher compared to HCl and HBr salts at higher pH values.

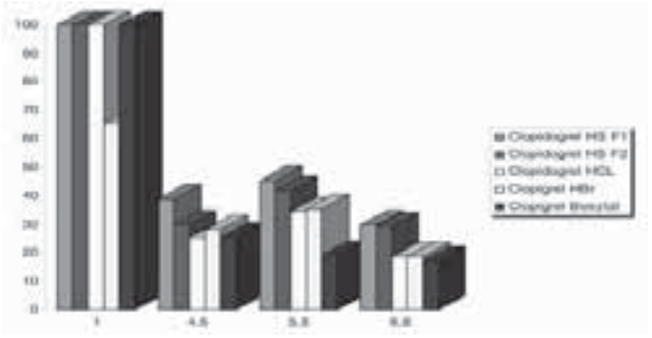

Figure 1: Comparison of different clopidogrel salts solubility in pH range 1-6.8.

From results it can also be established that besylate salt is less soluble than other salts especially at higher pH values. This can be estimated from solubility profiles.

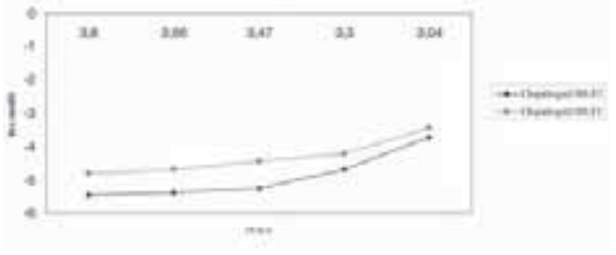

Figure 2: Solubility of clopidogrel HS F1 and F2 in acetone at different temperatures

Solubility of clopidogrel HS form 1 and 2 in acetone showed that at room temperature form 2 is less soluble than form 1. Experimentally determined mole fraction were linerized with respect to reciprocal values of temperature according to van't Hoff equition for ideal solutions (5). As shown in Figure2 experimental values for both polymorphs deviate from straight line, what is consequence of non-ideal behavior of solution. Solubility of form 1 is higher at lower temperatures compared to form 2. Nevertheless, if only solubilities at higher temperature are used for calculation, predicted solubilities from graph show, that it is reasonable to expect, that at enough high temperatures solubility of form 2 is higher then that of form 1.

If ideal behavior of solution could be presumed, transition point calculated from melting points and molar enthalpies of fusion using van't hoff equation would be at 421 K.

CONCLUSIONS

Solubilty of all clopidogrel salts is high at lower pH and strongly depends on pH value of aqueous solutions. Clopidogrel HS F1, F2 salts have slightly higher solubility at pH 4,5, water and pH 6,8.

Experimental data for solubility of HS F1 and F2 in acetone shows strong T dependence of solubility. Predicted solubilities from Figure 2 show, that it is reasonable to expect, that at enough high temperatures solubility of form 2 is higher then that of form 1.

References

1. Grant D.J.W., Theory and origin of polymorphism. V: Brittain H.G., Theory and Origin of Polymorphism, New York: Marcel Dekker, 1999: 1-33
2. D.A.Haynes, W.Jones, W.D.S.Motherwell., Occurrence of Pharmaceutically Acceptable Anion and Cations in the Cambridge Structural Database, J.Pharm. sc. Vol 94, NO.10,2005:2111-2120
3. Pereillo J-M, Maftouh M, Andrieu A, Uzabiaga M, Fedeili O, Savi P, Herbert J, Maffrand J, Picard C: Structure and stereochemistry of the active metabolite of clopidogrel. Drug Metabolism and disposition vol 30, No11 (2002); 1288-1295.
4. WO 2007/108604 A1 Pharmaceutical composition containing clopidogrel camphorsulfonate or polymorphic forms thereof. K.W.Jeoung, K.H. Kyong, S.K.Hyun. International Publication number. PCT WO2007/108604 A1.
5. Marin A: Physical Pharmacy, 4th ed., Lea &Feibiger, Phiadelphia, London, 1993: pp:33-34, 212-227.

PO152

Risk assessment approach for optimizing the equipment cleaning in penicillin dedicated production plant

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INTRODUCTION

Cleaning of equipment is highly regulated and controlled operation in the manufacturing of pharmaceuticals (1). It is a process which is removing physical, chemical and microbiological contaminants from the surface of manufacturing equipment (2,3). Good Manufacturing Practices require that the equipment must be always clean to the level that the quality, safety and efficacy of the next product/batch is assured. For the production of penicillin a dedicated equipment or completely separated facility is required as penicillin products belong to the high-risk products concerning the safety regarding their allergic adverse effects.

The prescribed cleaning procedure requires cleaning of equipment after each use, however, for dedicated equipment that kind of requirement is not so strict due to the same active ingredients (API) in the products and the same manufacturing process. Therefore, the modification and optimisation of the cleaning process is reasonable, possible and highly supported. In the dedicated manufacturing of penicillin products, which is a subject of our study, there is a single purpose production since it is intended only for the production of medicinal products containing amoxicillin and clavulanic acid. The production of film-coated tablets and powders for oral suspensions containing various quantities of amoxicillin is carried out according to the principles of dry technology and under controlled environmental conditions (RH <30 %). In the framework of this study an optimised cleaning procedure and the use of dedicated equipment,container in our case was designed with risk assessment approach (4). Risk assessment approach using FMEA (Failure Mode and Effect Analysis) for the evaluation of risks concerning quality is a systematic procedure for the identification of any possible potential failures with the intention of minimisation of these risks (5). On the basis of the risk analysis, the cleaning procedure among the individual products in a dedicated production may be optimised in such a way that the extent of cleaning can be reduced.

EXPERIMENTAL METHODS

For optimising the cleaning procedure, the basic optimisation criteria have to be considered. These have been determined using a decision tree and the process description (mapping). Implementation of FMEA was taking into consideration the analysis of possible errors in the cleaning procedure and their consequences with regard to cleanliness of the equipment. For the determination of API residues in the containers after using and before cleaning, a microbiological method for determination of antibiotics after sampling by the swab procedure (25 mm^2 area) from various sampling points has been used.

RESULTS

Criteria for studying the possibilities of cleaning optimisation were: i) type of equipment, ii) active substance, iii) product and iv) analytical method. The container was using as a representative equipment, active substance was amoxicillin and granules for tablets or powders for oral suspensions with the highest quantity of amoxicillin have been used as representative products. The diffusion of antibiotic in agar has been used as an appropriate analytical method.

Process mapping for individual representative products has been carried out with a detailed examination of the technological phases. Within FMEA, the risks for the formulations were defined (using granules and powders) with regard to their possible residues (including API and the excipients) on non-critical and critical points of container. The fundamental risk was associated with the possibility of i) increased quantity of API and ii) increased quantity of excipients in the next batch product and additionally iii) the possibility of the microbiological contamination. The corrective measures to reduce the risk have been evaluated, i.e. visual examination of the of the container s interior :no residues of powders and granules should be present, the valve should be empty. It has been decided that on the critical point, e.g. packing ring (sealing), vacuuming is required. The maximum number of containers for carrying out the optimised cleaning procedure was also defined. On the basis of the amoxicillin residue in the container after three consecutive cycles of usage on various sampling points: i)inner surface of the container s lid, ii) container s interior surface , iii) inner side of the container ceiling, iv) surface of the outlet valve, v) screws near the ring of the outlet valve and vi) the ring of the outlet valve - the residue was calculated for particular formulation (granules and powder (Figure 1).

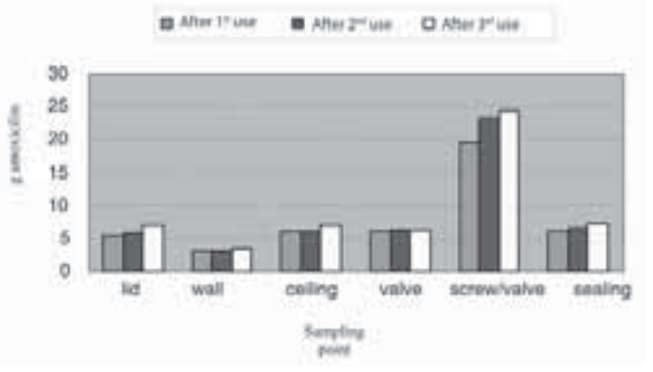

Figure 1: The residue of amoxicillin after three consecutive uses of the same container (granules case)

Table 1: Theoretical residue of amoxicillin in consecutive tablets batch (different doses of API)

Residue 24,3 g Consec. batch	1 g	625 mg	375 mg	562,5 mg
Increase of API.	0.012%	0.016%	0.019%	0.014%

The hypothetical (computed) residue of amoxicillin per entire surface of the container (Table1) was calculated by using the highest quantity of amoxicillin found on the container s screw /valve place (Tab.1). This has been considered as the worst case.

Taking into account that the real amount of residue is located on other 99% of the entire surface , than the real amoxicillin residue was recalculated according to the container s “wall data” (Fig.1, Table 2)

Table 2: Real residue of amoxicillin in consecutive tablets batch (different doses)

Residue 4,1g cons.batch.	1g	625 mg	375 mg	562,5 mg
Increase of API	0.0019%	0.0027%	0.0033%	0.0030%

CONCLUSIONS

After using of the risk analysis methodology it has been estimated that amoxicillin residue in the successive product batches is below 0.01%, what is significantly under the regulatory permitted variations of the API content in the finished product (5%). It was additionally concluded that the residue of the excipients does not have any negative impact on the quality of the finished products (bellow 0.005%). The risk of microbiological contamination is not increased as these products contain penicillin and are manufactured under the strictly controlled manufacturing environment (i.e. RH below 30%).

REFERENCES

1. William E. Hall: Cleaning in the pharmaceutical industry- past, present, and future; J. of Valid. Technol., **14** 2007: 42-48
2. Jenkins KM, Vanderwielen A J. Cleaning validation: An overall perspective. Pharm. Technol. **18**, 1994: 60-12.
3. Fourman, G.L. and Mullen, M.V., "Determining Cleaning Validation Acceptance Limits for Pharmaceutical Manufacturing Operations," Pharm. Technol.; **17,**1993, 54-60
4. James L. Vesper: Risk assessment and risk management in the pharmaceutical industry, PDA/DHI; 2006 ,10-23.
5. D. W. Vincent and B. Honeck: Risk Management Analysis Techniques for Validation Programs, J. of Valid. Technol.; **10,** 2004: 235-251.

PO153

Influence of luminal monosaccharides on permeability of glutathione conjugates across rat small intestine *in vitro*

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INTRODUCTION

MRP (multidrug resistance associated protein) transporters are a well-known subfamily of efflux ABC transporters. Since detoxification is the basic function of efflux transporters, MRP2 transporter is one of the most important. It resides on the apical side of epithelia in eliminatory organs such as the liver, kidneys and intestine and is facilitating drug secretion into the bile, renal tubules and intestinal lumen. Substrates include xenobiotics such as anticancer and anti-HIV drugs and endogenous substances such as biliary acids and glutathione (GSH) conjugates which are commonly used as markers for MRP activity [1]. On the other hand, GSH conjugates are also substrates for a non-MRP transporter RLIP76. It is not a member of ABC transporter family but shares their tissue distribution and substrate specificity.

MRP activity is under constant translational, transcriptional and post-transcriptional control. The posttranscriptional level includes vesicular trafficking and allosteric regulation. Cellular trafficking of vesicles containing MRP transporters is well documented in the liver and in the kidneys. It affects the amount of transporters in the cellular membrane by regulating rates of exocytotic incorporation of transporters into the apical or basolateral membrane and their endocytotic retrieval into the intracellular pool of vesicles. Although MRP transporters are also expressed in the small intestine where they can influence the pharmacokinetics of their substrates, no research at all has been done on cellular trafficking of MRP transporters in the intestinal mucosa [1].

The aim of the present study was to investigate the regulation of MRP activity in the rat small intestine *in vitro* using GSH conjugates.

MATERIALS AND METHODS

S-(2,4-dinitrophenyl)glutathione (DNPSG) was synthesized as described by Hinchmann et al. 1-chloro-2,4-dinitrobenzene (CDNB) and all other chemicals were purchased from Sigma and were of highest purity grade available. N-ethylsuccinimido-S-glutathione (NEMSG) was prepared by an *in situ* reaction between N-ethylmaleimide and GSH.

Experiments were performed as previously described [2]. Shortly, rat intestine from male Wistar rats was mounted in EasyMount Sweetana-Grass type side-by-side diffusion chambers.

Mucosal solutions consisted of 1 mM acivicin, inhibitor of GSH degradation, and 10 mM of appropriate monosaccharide in Ringer buffer. Serosal solutions contained 10 mM glucose (GLU) in Ringer buffer. The concentrations of DNPSG and NEMSG in the donor solutions were 1 mM while CDNB was used in a 0.1 mM concentration.

Tissue viability and integrity were monitored by electrophysiological parameters throughout the experiments. Segments with inappropriate viability were excluded from statistical analysis.

All analytes (CDNB, DNPSG and NEMSG) were analyzed by HPLC. All data are presented as excretion ratio (R_{ex}) ±SEM. R_{ex} is defined as a quotient of permeability coefficients from serosal to mucosal (M-S) and from mucosal to serosal (M-S) compartment.

RESULTS AND DISCUSSION

Permeability experiments with DNPSG in S-M and M-S directions were performed with different monosaccharides on mucosal side of the intestine. R_{ex} values of the performed experiments are shown in figure 1.

Figure 1: Effects of monosaccharides on R_{ex} value of DNPSG

To investigate how monosaccharides influence DNPSG permeability, we focused on the metabolic processes that take place when a specific monosaccharide comes in contact with enterocytes. GLU, for instance, is transported by SGLT and apical GLUT transporters into enterocytes and exits them through basolateral GLUT transporters. Inside the cells it can also be phosphorylated and metabolized to yield energy. Most of these processes or metabolites (e.g. phosphorylated products) can stimulate metabolic responses. To elucidate which process is essential for the change in DNPSG permeability, we have used GLU, mannitol (MAN), fructose (FRU), α-methylglucopyranoside (MeGLU) and galactose (GAL). These monosaccharides have metabolic paths different from GLU or share just some common properties. In table 1, these transport properties of used monosaccharides are shown.

Table 1: Transport properties of monosaccharides and Rex values

	MAN	GLU	FRU	MeGLU	GAL
SGLT	**NO**	**YES**	**NO**	**YES**	**YES**
GLUT	NO	YES	YES	NO	NO
phospho rylation	NO	YES	YES*	NO	YES*
energy	NO	YES	YES	NO	YES
R_{ex}	**1.04**	**1.88†**	**1.02**	**1.54†**	**1.94†**

* - can be phosphorylated but yields a different metabolite
† - R_{ex} value is larger then unity ($p<0.05$)

By comparing experimental results (R_{ex}) with metabolic properties of monosaccharides, we can see that secretion of DNPSG is triggered by binding to or transport by SGLT transporters, most probably SGLT1 or SGLT3. Both subtypes are expressed in the intestine and can be involved in different epithelial responses, because SGLT transport is electrogenic and can depolarize the membrane [3].

It is known that GLU in the gut can trigger many epithelial responses through SGLT transport. This can change passive permeability (by opening tight junctions or by acidification of mucosa) and altering the transporter-mediated permeability of charged compounds. To examine mechanism of SGLT influence, we performed experiments with two additional markers (NEMSG and CDNB).

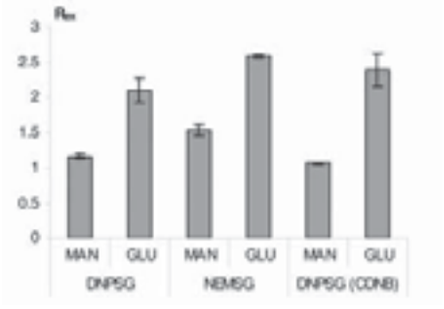

Figure 2: Effect of MAN and GLU on R_{ex} value of the markers

Results of experiments with NEMSG and CDNB are very similar to those obtained by DNPSG experiments. R_{ex} values are larger then unity ($p<0.05$) only when GLU was present on mucosal side.

DNPSG and NEMSG are GSH conjugates with similar physicochemical properties. They have a comparable size, are zwitterionic and highly hydrophilic. CDNB, on the other hand, is uncharged at all physiological conditions, has much lower molecular weight and is lipophylic. CDNB is not MRP substrate, but a precursor of DNPSG. As seen on figure 3, CDNB readily passes cellular membrane and inside the enterocytes it conjugates with GSH. Subsequently, intracellular pool of DNPSG is formed and the conjugate permeates or is transported out to serosal or to mucosal side of the tissue.

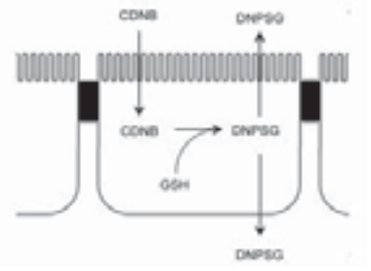

Figure 3: Metabolic fate of CDNB in enterocytes

Since the results obtained with different markers are very similar, we can assume that the effect of GLU on the permeability of GSH conjugates is not caused by a change in permeability of tight junctions or by acidification of luminal mucosa, but by a more specific process. That is most probably an increase in MRP2 or RLIP76 transporter activity.

CONCLUSION

GLU in the gut triggers the efflux of GSH conjugates. Most probable metabolic process which triggers the efflux is binding to or transport with SGLT transporters. Since GSH conjugates are substrates of MRP transporter family and RLIP76 transporter, the efflux is most probably related to a change in their activity. Further research will be needed to determine the subtype of triggering SGLT, the responding efflux transporter, the intracellular pathway between them and the physiological purpose of this connection.

References

1. Gerk PM, Vore M: Regulation of Expression of MRP2 and Its Role in Drug Disposition, JPET, 302(2): 407-415 (2002)
2. Žakelj S, Legen I, Veber M, Kristl A: The influence of buffer composition on tissue integrity during permeability experiments “in vitro”, Int. J. Pharm, 272: 173–180 (2004)
3. Nusrat A, Turner JR, Madara JL: Molecular Physiology and Pathophysiology of Tight Junctions IV. Regulation of tight junctions by extracellular stimuli: nutrients, cytokines, and immune cells, Am J Physiol Gastrointest Liver Physiol 279: G851-G857 (2000)

PO154

Determination of biogenic amines in human plasma with HPLC method and electrochemical detector

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INTRODUCTION

Biogenic amines are important group of endogenous molecules which act as neurotransmitters or hormones. Their quantification in physiological fluids has an established role in clinical chemistry as these molecules serve as diagnostic markers for variety of metabolic and neurological disorders. The neurotransmitters undergo extensive metabolism in the central nervous system. Plasma concentrations of their end-metabolites i.e. 3-methoxy-4-hydroxyphenylglycol (MHPG), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), and 5-hydroxyindoleacetic acid (HIAA), are frequently used as markers of noradrenalin, dopamine, and serotonin metabolism, respectively (1,2).

The concentrations of biogenic amines in plasma samples are in nanomolar range. Therefore a sensitive analytical methods have to be employed in order to obtain suitable method used for sample quantification. In particular, high performance liquid chromatography (HPLC) coupled with electrochemical detector (ECD) has provided a sensitive tool to determine the low levels of these compounds (1).

The aim of the present work was to develop a sensitive method for quantification of MHPG, HVA and HIAA in plasma samples using HPLC with ECD. The method is intended for determination of analytes in plasma samples of patients with schizophrenia. Some preliminary results of the method development are described herein.

METHODS

Chromatographic system

The HPLC system Agilent 1100/1200 series (Agilent Techologies Inc.) consisted of the qauternary pump, vacuum degasser, column thermostat (35 °C), autosampler (5 °C) and UV detector. Additionally a CoulArray Model 5600 (ESA Inc) electrochemical detector was coupled to the system after UV detector using the valve switching device. The chromatographic separation was obtained with a C18 110A reversed-phase column (Phenomenex Gemini, 50 x 4.6 mm, 5 γm) that was connected to the precolumn. The mobile phase consisted of the solvent A (50 mM KH_2PO_4 pH= 3.0) and solvent B (98/2 acetonitrile/water). The flow rate of the mobile phase was 1 mL/min. An 50 μL injection of sample was loaded onto the column, separated and eluted using the following gradients (minutes, % mobile phase B) (0, 2) (3, 2) (3.1, 5) (14, 5) (14.1, 2) (20, 2). The analytical cell of ECD consisted of four serial carbon porous electrodes and optimal potentials of 500, 0, 0 and -400 mV were applied on the 1st-4th electrode, respectively. The electrochemical responses of all analytes were measured on the first (oxidation) and fourth (reduction) electrode. The column system was connected to ECD, except for the time period from 0.5 to 2.3 minutes after the sample injection. The HPLC system was controlled by an HP ChemStation for LC, Rev.A.06.03, except for the CoulArray electrochemical detector, which was controlled by ESA CoulArray for Windows 1.04.

Solid phase extraction (SPE) procedure

The analytes were extracted from samples using SPE cartridges (Strata-X 33μm, 30 mg; Phenomenex Inc.) and vacuum manifold connected to the oil aspirator. The steps of SPE procedure were the following:

- conditioning with 2 ml of methanol,
- equilibration with 1ml of 50 mM KH_2PO_4 pH 5.5,
- loading of 0.5 ml of plasma sample and 1 ml of 50 mM KH_2PO_4 pH 5.5,
- washing with 2 ml of 50 mM KH_2PO_4 pH 5.5 and
- elution with 1 ml of methanol.

The eluate was dried out at 40°C under a gentle stream of nitrogen in a Turbovab apparatus (Zymark Inc.). The dried samples were reconstituted in 200 L of the 50 mM KH_2PO_4 with 1 mM EDTA pH 3.0 prior the injection.

Standard solutions

The stock solutions of analytes and homovanillic alcohol used as internal standard (IS) were prepared in 50 mM KH_2PO_4 with 1 mM EDTA pH 3.0. The standard solutions were prepared daily fresh in artificial plasma (2.2 mg KCl, 88 mg NaCl, 2.2 mg KH_2PO_4, 2.6 mg Na_2HPO_4, 4.1 mg EDTA, and 444 mg bovine serum albumin in 10 ml of water). The standard samples used for calibration and quality control samples contained analytes in the concentration range from 1 to 90 μg/L.

RESULTS AND DISCUSSION

The electrochemical behaviour of the analytes enables substances to be oxidized. Generating oxidation products can then be reduced at the following electrode. To obtain such a conditions appropriate potentials have to be applied on the electrodes (Fig.1). Therefore oxidation with the following reduction of the analytes can be used for their measurements.

The chromatographic condition enabled separation of analytes and in case of HVA appropriate selectivity was obtained when its oxidised product was specifically reduced at -400 mV (Fig.2).

According to the FDA guidance the HPLC method was partially validated (Table 1). The validation parameters were within the prescribed limits.

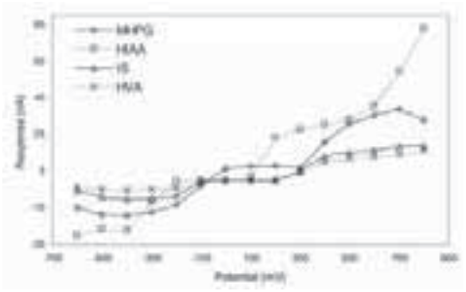

Figure 1: Hydrodynamic voltammograms of MHPG, HIAA, IS and HVA. The concentration of analytes was 40 nmol/l.

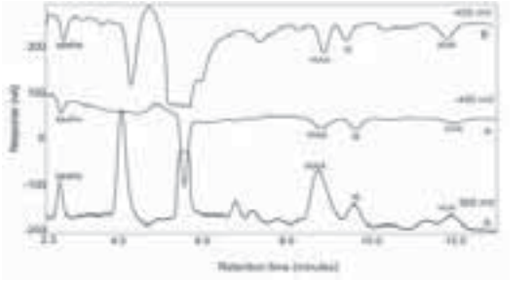

Figure 2: (A) Chromatograms of artificial plasma sample obtained at 500 and -400 mV. The sample was spiked with approximately 8.5 μg/L of analytes. (B) Chromatogram of plasma sample obtained from healthy subject.

Table 1: Summary of method validation parameters.

Validation parameter		*MHPG*	*HIAA*	HVA
Intraday accuracy	QC_l	103.2 (7.8)	112.3 (6.8)	115.3 (11.7)
and precision (RSD)	QC_m	102.7 (4.1)	95.9 (7.3)	95.5 (5.0)
in %; n=3	QC_h	97.7 (2.0)	108.8 (5.2)	97.4 (2.0)
Extraction recovery	(%)	97.3	79.3	103.5
Detection limit (μg/L)		0.50	0.90	0.88
Linear range in μg/L		1.4 – 54.5	1.5 – 59.9	1.5 – 59.4

RSD, relative standard deviation; QC_l, QC_m, and QC_h stand for low, medium, and high quality control plasma samples, respectively.

CONCLUSION

The SPE method combined with liquid chromatography with ECD was developed and partially validated. The method enabled simultaneous measurements of MHPG, HIAA and HVA. The appropriate method selectivity was achieved with the reversible oxidation and reduction of the analytes. The obtained sensitivity of the method should be appropriate for analytes quantification in the plasma samples of patients with schizophrenia.

References

1. Unceta N, Rodriguez E, de Balugera ZG *et al.* Determination of catecholamines and their metabolites in human plasma using liquid chromatography with coulometric multi-electrode cell-design detection. Anal Chim Acta, 2001; 444:211-221.
2. Kestell P, Zhao L, Jameson MB *et al.* Measurement of plasma 5-hydroxyindoleacetic acid as a possible clinical surrogate marker for the action of antivascular agents. Clin Chim Acta, 2001; 314:159-166.

PO155

Comparative study of physical performance of formulations containing antihypertensive drug alone and in a combination with diuretic

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INTRODUCTION

Valsartan is a nonpeptide, orally active, and specific angiotensin II antagonist acting on the AT1 receptor subtype (1). It is known that valsartan could be applied alone or in combination with other active ingredients, most often with a thiazide diuretic hydrochlorothiazide (2). Valsartan and hydrochlorothiazide are only slightly soluble in water (1). Despite their low solubility, the dissolution for both active ingredients could be improved by incorporation of a suitable amount of a specific superdisintegrant croscarmellose sodium and not by particle size reduction as one of the most common methods for improwing solubility and dissolution rate (3,4).

Superdisintegrants differ from traditional disintegrants in that they are effective at much lower concentrations. Because of the complexities involved, the exact mechanism of action of superdisintegrant is not well understood. Some of the proposed mechanisms include water wicking, swelling, deformation recovery, particle repulsion, and heat of wetting. Water uptake is necessary precursor to all other mechanisms (4).

The aim of our study was to compare physical performance of formulations containing valsartan alone and in a combination with hydrochlorothiazide and evaluation of impact of croscarmellose sodium content and particle size of valsartan on dissolution characteristics.

EXPERIMENTAL METHODS

Composition and technological process of preparation of film coated tablets containing valsartan

Tablets, containing valsartan, microcrystalline cellulose, lactose monohydrate, povidone, croscarmellose sodium, colloidal anhydrous silica, magnesium stearate and coating layer, comprising hypromellose, macrogol 4000, E171 and optionally E172, were included in the study. The strenghts 40 mg, 80 mg, 160 mg and 320 mg were in linear proportion for the compositon of the core with minor difference in the composition of the coating layer for easier differentiation. Valsartan was incorporated into granulate by wet aqueous granulation according to the process, described in (3).

Composition and technological process of preparation of film coated tablets containing valsartan and hydrochlorothiazide

Tablets, containing valsartan, hydrochlorothiazide, microcrystalline cellulose, lactose monohydrate, povidone, croscarmellose sodium, colloidal anhydrous silica, magnesium stearate and coating layer, comprising hypromellose, macrogol 4000, E171 and optionally E172, were included in the study. The amounts of valsartan 80 mg, 160 mg and 320 mg were combined with 12.5 mg and 25 mg of hydrochlorothiazide in the following strenghts: 80/12.5 mg, 160/12.5 mg, 160/25 mg, 320/12.5 mg and 320/25 mg. Valsartan was incorporated into granulate by wet aqueous granulation according to the process, described in (3).

Physical characteristics

Physical characteristics of granulate and compression mixture were determined:

- flowability and angle of repose on apparatus Pharmatest PTG,
- bulk and tapped volume on apparatus Engelsmann.

Dissolution profile determination

Dissolution profiles were performed using basket apparatus in phosphate buffer solution.

RESULTS AND DISCUSSION

Wet aqueous granulation is advantageous process over direct compression due to the non-optimal physical characteristics and high content of valsartan in the formulation (>30%). In the study of the influence of croscarmellose sodium concentration onto dissolution of valsartan from tablets containing 80 mg of valsartan, inversely proportioned relationship was obtained, meaning that incorporation of lower concentration of croscarmellose sodium (1%, formulation A) resulted in faster dissolution in comparison to the formulation with higher concentration (5%, formulation B). Lower value value for bulk volume of granulate in formuation B is attributed to higher amount of croscarmellose sodium, which acts also as a binder and connects particles in the granulate stronger because of higher number of interparticulate physical bonds (Table 1).

Table 1: Physical characteristics of granulate.

Parameter	Formulation A	Formulation B
Flowability [s]	17.9	17.3
Angle of repose [°]	32.1	33.0
Bulk volume [mL/g]	2.26	2.02
Tapped volume [mL/g]	1.82	1.60

Faster initial dissolution of valsartan was obtained in formulation with lower croscarmellose sodium concentration (formulation A) in comparison to formulation with higher superdisintegrant content (formulation B) (Figure 1).

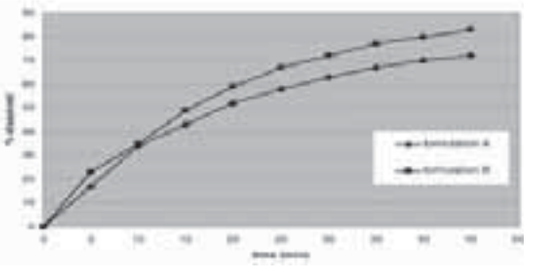

Figure 1: Dissolution profiles of formulations A and B.

No significant difference has been observed in dissolution of formulations containing two different particle size of valsartan in formulation containing a combination valsartan/hydrohlorothiazide 320/25 mg (formulation C: average particle size <50 μm; formulation D: average particle size >100 μm) (Figure 2).

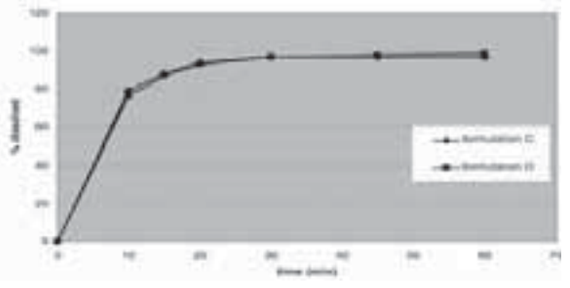

Figure 2: Dissolution profiles of formulations C and D.

CONCLUSIONS

The results of our study have proven that lower concentration of a specific superdisintegrant croscarmellose sodium in formulations containing valsartan alone or combined with hydrochlorothiazide results in faster initial dissolution than in the case of higher concentration, although technological characteristics and performance of the process were similar. Furthermore, no significant influence of particle size of valsartan was observed if using two different average particle size of valsartan, i.e <50 μm and > 100 μm. An extragranular incorporation of hydrochlorothiazide does not have a significant influence on dissolution rate and profile of valsartan.

The optimized formulation with lower amount of croscarmellose sodium enables performing compression of the mixture at low main pressure in tabletting process (<15 kN). This finding attributes to higher speed of tabletting, which is advantageous in industriability, reproducibility and processability of the said formulation.

References

1. www.rxlist.com
2. www.drugs.com
3. WO2006/066961
4. Swarbrick J. Encyclopedia of pharmaceutical technology, 3rd ed. New York, Informa Healthcare, 2007.

PO156

The influence of plasticizer addition on wetting properties of polymer coating dispersion

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INTRODUCTION

In the pharmaceutical industry, polymeric films are generally applied to solid dosage forms using a spray-atomization technique. The polymer is dispersed in aqueous or organic solvents prior to spraying. The coating dispersion is atomized with air into small droplets, which are then delivered to the surface of the substrate. Upon contact, the atomized droplets spread across the substrate surface. As the solvent begins to evaporate, the polymer particles densely pack on the surface of solid. The time required to obtain a stable film without further aging effects is dependent on a number of factors, including the type and concentration of plasticizer (1).

Many pharmaceutical polymers exhibit brittle properties and require the addition of a plasticizing agent to obtain an effective coating, free of cracks, edging, or splitting. Plasticizers function by weakening the intermolecular attractions between the polymer chains and generally cause a decrease in tensile strength and the glass transition temperature and in an increase in the flexibility of the films (1).

The objective of this study was to investigate the influence of the amount of plasticizer on wetting properties of polymer coating dispersion. Properties such as contact angle and wetting rate on compressed microcrystalline cellulose were studied. In order to obtain smooth coated tablets or pellets and to lower the risk of peeling-off during process of film coating small contact angle (good wetting) and fast wetting rate of polymer dispersion on substrates surface are desirable.

EXPERIMENTAL METHODS

Materials: Kollicoat® SR 30D (BASF Corporation, Germany) was used as model polymer dispersion. Triethyl citrate (TEC) was used as plasticizing agent in amounts of 0 wt% (no TEC added), 1 %wt, 5 wt% and 10 wt%.

Microcrystalline cellulose (Avicel® PH 105, FMC Corporation, USA) has been compressed with 150 kN force into the plates (weight 0,3g, dimension 1,0 cm x 2,5 cm). *Contact angles* of polymer dispersion on compressed microcrystalline cellulose were measured by Drop Shape Analysis System - DSA 100 (Krüss, Germany). Contact angles were measured on static drops. The drop of volume 2 μL is produced and spreaded before the measurement takes place. Contact angles were determined by Young-Laplace (sessile drop) fitting (2). 30 measurements were made for each series to obtain mean contact angle value.

Dynamics of wetting was determined by following the contact angle of a drop during spreading on a solid surface. Through the use of the residuals we could numerically estimate the rate of spreading the drop (Fig. 1). The slope of a function Ln(residual value)/time represents the rate of wetting.

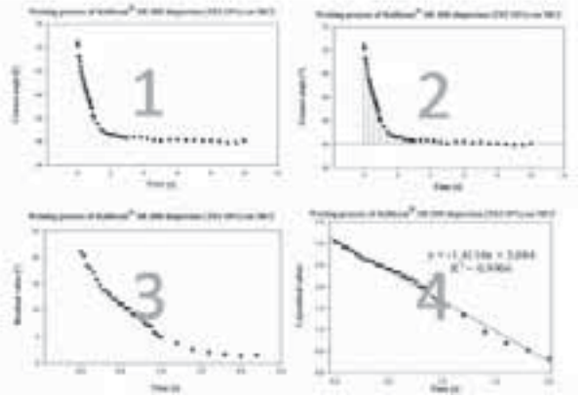

Figure 1: The use of the residuals for numerical estimation of the wetting rate.

Viscosity of the polymer dispersion was measured at room temperature by capillary Cannon-Fenske viscometer (K=0,2380 mm^2/s^2, Schott-Geräte, Germany).

RESULTS AND DISCUSSION

Contact angles of Kollicoat® SR 30D dispersions with different amount of plasticizer TEC on compressed microcrystalline cellulose are reported in Fig. 2. As can be seen, the addition of 1 wt% TEC improves the wetting of the substrate. There is a clear trend of worsening the wetting with further plasticizer inclusion into polymer dispersion.

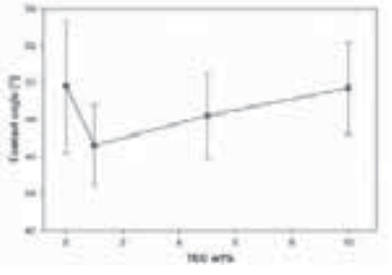

Figure 2: Static contact angles of Kollicoatâ SR 30D dispersions on microcrystalline cellulose.

Wetting dynamics of polymer coating dispersions with different amounts of plasticizer TEC on compressed microcrystalline cellulose are shown in

Fig. 3 and Table 1. It is demonstrated that the inclusion of plasticizer into the polymer dispersion decrease evidently the wetting rate.

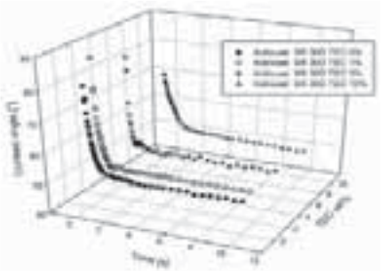

Figure 3: Wetting dynamics of polymer coating dispersions on compressed microcrystalline cellulose.

Table 1: Numerical values of wetting rates of polymer coating dispersions with different amounts of plasticizer.

TEC addition (wt%)	Wetting rate (s^{-1})	R^2
0	3,1	0,995
1	2,8	0,976
5	2,1	0,737
10	1,4	0,991

The effect of plasticizer TEC addition on Kollicoat® SR 30D dispersion viscosities was studied (Fig. 4). There was a noticeable increase of viscosity of polymer dispersion which could be indicative of extensive plasticizer-polymer interaction. Plasticizer molecules diffuse between polymer chains causing water molecules to be driven out from polymer chains and thus increased viscosity of polymer dispersion is measured (3).

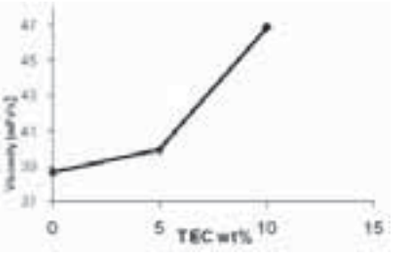

Figure 4: Effect of plasticizer inclusion on polymer coating dispersion viscosity.

CONCLUSION

Wetting properties of Kollicoat® SR 30D confirmed the differences among dispersions when different amounts of plasticizer TEC have been used. Inclusion of 1 wt% of plasticizer seems to be optimal for coating process due to the lowest contact angle and relatively good wetting dynamics on substrates surface. Wetting rate is significantly influenced by the amount of included plasticizer and with the polymer dispersion viscosity.

References

1. Felton LA. Film coating of oral dosage forms in Swarbrick J., Boylan CJ. Encyclopaedia of Pharm. Technology, 3rd Ed., New York: Informa Healthcare, 2006; 1729-1747
2. Rotenberg Y, Boruvka L, Neumann AW. J. Colloid Interface Sci. 1983, 93, 169.
3. Martin A. Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences, 4th Ed. Philadelphia, 1930, 580-590

PO156

Micro-raman spectrometry for detection of local polymorphic forms in solid pharmaceuticals

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INTRODUCTION

API molecules of different conformation or packing establish polymorph, solvatomorph, desolvated crystalline solvate or amorphous forms of different types. The changes of enantiotropic materials between forms, being stabile at different temperatures, are governed by thermodynamic control [1]. In amorphous materials, however, the changes of arrangement and molecular mobility at glass transition temperature (T_g) are controlled kinetically.

The morphology of drugs may influence their processability to solid dosage forms and also the bioavailability substantially [2]. Furthermore the influences of various steps of the technology may alter the original forms of API-s. Thus the sophisticated chemical processes applied for synthesizing certain polymorphic or amorphous forms are useless if these can not be preserved during the subsequent processes of granulation, tablet forming, coating. Solvatation, desolvatation, amorphization, crystallization or transcrystallization may occur due to the influence of shear, temperature, compression and interaction with other ingredients [3]. As these influences are hardly controlled in the current technologies urgent need exists, at each stages of formulation process, for (preferably in line) analyses being able to determine the local changes of the structure of pharmaceuticals that may influence the efficiency and safety.

Solid-phase analytical methods being applicable for the characterization of the morphology of an API include WAXS, vibrational spectroscopy, solid phase NMR, thermal and optical analyses [4]. The IR and Raman microscopy is suitable to provide the two dimensional chemical image of objects. The spatial resolution of IR and dispersive Raman microscopes are about 10 μm, and 1 μm respectively. Raman micro-spectrometry allows to determine the local structure of components in multicomponent systems advantageously [5]. Solid phase NMR (ss-NMR) (which excites the sample with series of magnetic impulse and provides spectra of the Fourier-transformed relaxation signal) became valuable tool in the analysis of polymorphism recently. The method is applicable not only to distinguish the different polymorphic forms from each other but direct structural information can be gained too. Spectroscopy of Thermally Stimulated depolarization Current (TSC) is a unique tool for getting an insight in the structure of amorphous materials. Changes of mobility of groups and other molecular units at certain temperatures can be detected sensitively, therefore not only the thermodynamic transitions but also the kinetically controlled changes can be monitored this way [6].

This paper presents new spectroscopic ways for in line and off line structural analysis of solid pharmaceuticals at various stages of technology.

EXPERIMENTAL METHODS

The chemical structures were investigated by micro-Raman analysis using a LabRam type Jobin Yvon apparatus with frequency doubled NdYAG laser of 532 nm, power: 10 mW without intensity filter, the objective: 50x.

RESULTS

Local arrangement of API in the neighbourhood of various excipients of granules was examined. In the case of the example shown in this paper the excipient was lactose monohydrate. Figure 1 shows the optical image of the analyzed area.

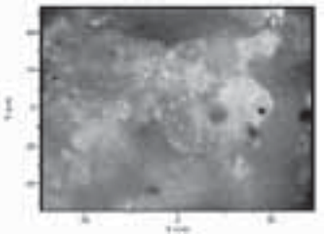


Figure 1: Optical microscopic image of granule part

The Raman image shows the arrangement of the API (bright) in the neighbourhood of lactose monohydrate.

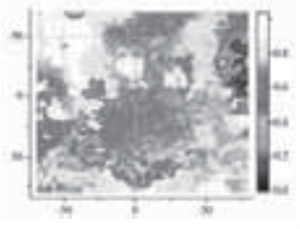
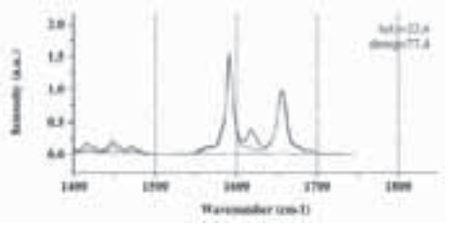


Figure 2 Chemical mapping image showing the main polymorph of API (bright areas)

The mapping indicated the presence of another polymorph in a very low concentration at certain areas.



The mapping allowed determining the local arrangement of the traces of minor polymorph.

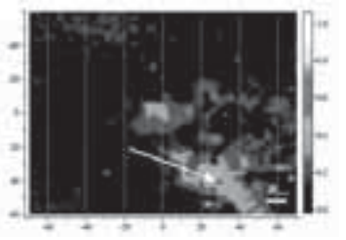


Figure 4: Sites where minor polymorph (coloured areas) was detected

The method was used for accelerated analysis of polymorph stability and in line control of coating process too.

CONCLUSIONS

Raman microscopic imaging allowed us to detect the polymorphic impurities of API in multicomponent pharmaceutical product at such a low concentration that could hardly be detected by any other technique. The fibre optic detector of the system allowed us to use the system for in line PAT purposes.

ACKNOWLEDGEMENT

The authors acknowledge the financial supports received through the EU-6 Framework Program (NMP3-CT-2004-505637), Multihybrids (IP 026685-2) and Public Benefit Association of Sciences and Sport of the Budapest University of Technology and Economics.

References

1. D.J.W. Grant, *Theory an origin of polymorphism*, in: H.G. Brittain (Ed.): *Polymorphism in Pharmaceutical solids*, Vol. 95, Marcel Dekker, New York, 1999, p. 1-33
2. J.K. Haleblian, W.C. McCrone, *Pharmaceutical applications of polymorphism*, J. Pharm. Sci. 58, (1969) 911-929
3. H.G. Brittain, E.F. Friese, *Effects of pharmaceutical processing on drug polymorphs and solvates*, in: H.G. Brittain (Ed.), *Polymorphism in pharmaceutical solids*, Vol. 95, Marcel Dekker, New York, 1999, p.331-361
4. G.A. Stephenson, R.A. Forbes, S.M. Reutzel-Edens, *Characterization of the solid state: quantitative issues*, Adv. Drug Deliver. Rev. 48 (2001) p. 67-90
5. Pelletier, M. J., *Quantitative Analysis Using Raman Spectrometry*, Appl. Spectr.. 57 (2003) 1
6. N.T. Correia, J. Moura, M. Descamps, G. Collins: *Molecular mobility and fragility in indomethacin: a Thermally Stimulated Depolarisation Currents study*, Pharm Res. 18 (2001) 1767–1774

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