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SLOVENIAN PHARMACEUTICAL SOCIETY
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Slovenian pharmaceutical society (sfd)

Slovenian Pharmaceutical Society is the most important link between different segments of the Slovenian pharmaceutical community which is far more heterogeneous as in countries where the majority of pharmacists practice their profession in pharmacies. In Slovenia only 50 % of pharmacists are employed in pharmacies, most of the others in pharmaceutical industry. A number of connections have been built on different levels among pharmacists employed in industry, pharmacy services, educational and scientific institutions and administrative services.

The Slovenian Pharmaceutical Society was founded at a meeting of the Slovenian pharmacists summoned on 25 and 26 February 1950. The first decade of the Society's exisitance was of major importance for the later foundation of the Institute of Pharmacy and Drug Control in 1955 and for the introduction of a complete university study course in pharmacy in 1961; a decision that at the beginning did not find support even among the professional colleagues. At that time a unifying process already went on among the Slovenian pharmacists on regional and professional principles. Pharmacists from the same region founded subsidiaries, on the other hand a number of connections have been built on the professional level amoung pharmacists employed in pharmacy services, technologists, scientists… At the present time members of the Society are connected in 9 subsidiaries and in 11 sections (Section of Pharmaceutical Sciences, Senior Section of Pharmaceutical Technicians, Medicinal Chemistry Section, Section, Students' Section, Technological Section, Section of Clinical Pharmacists, Section of Hospital Pharmacists, Community Pharmacists' Sections, Regulatory Section and Homeopathic Section).

After 1971, in the period of speicalized-staff leveling, the importance of the Society began to grow and the first symposium, dealing with drug interactions, was held at the occasion of the Society's regular annual assembly. Since then, more than 50 symposia took place, many of them of international character. These professional events have developed into a regular activity of the Society, especially after 1991 when Slovenia proclaimed its independence.

From then, many prominent international experts have been invited to present plenary lectures at the Society's Symposia. Among these, distinguished representatives of pharmaceutical and health organizations such as FIP, WHO, EuroPharm Forum, European Pharmacopoeia, Regulatory bodies of European Community.

The Society's activity on the national and international level is reflected in its membership or the membership of its sections in a number of Associations, such as the FIP, EUFEPS, EAHP, ESCP, EPSA, IPSF, etc.

4 years ago the Slovenian Pharmaceutical Society founded the *Pharmaceutical Information Center* whose aim is to support safe, effective and efficient use of medicines. The service is providing information about new drugs and advices for clinical problems to pharmacists, members of the Society. Informations are published on the web side www.sfd.si and distributed by e-mails. The service supports also the pharmaceutical care of individual patients.

Very soon after its foundation the Society began with the publication of the Pharmaceutical Journal of Slovenia - *Farmacevtski vestnik* which has been regularly publishing up to the present. The initial number of 400 issues grew to the present 3.200, parallel to the increasing number of the Society's members and the number of other Journals interchanged for Pharmaceutical Journal.

The present, special issue of the Pharmaceutical Journal of Slovenia - *Farmacevtski vestnik* is dedicated to the promotion of Slovenian Pharmaceutical Society, especially the Section of Pharmaceutical Sciences at the 2nd PharmSciFair in Nice. The most important scientific achievements from different fields of pharmaceutical science are presented in the manner of abstracts of the most important publications in the last three years. The abstracts are divided under different topics, although the large number of publications shows the interdisciplinary character of pharmaceutical science. I sincerely thank all the contributors for their material.

Assist. Prof. Dr. Aleš Obreza, Guest Editor

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University of Ljubljana, Faculty of Pharmacy

Faculty of Pharmacy at the University of Ljubljana is a modern educational and research facility that combines the most up to date approaches of pedagogical and scientific research work. Innovations introduced in the high education institutions and scientific research laboratories by the most developed faculties in the modern world are also reflected in the development strategy of the Faculty of Pharmacy in Ljubljana.

The history of undergraduate study of pharmacy in Ljubljana began in 1946. The study programme of pharmacy was carried out in the first purpose-built building, which was designed by architect Ple nik in 1921. After the reorganization of study programmes, the comprehensive study programme in pharmacy was introduced in 1960 at the Faculty of Natural Sciences. In 1995, after the reorganization of the Faculty of Natural Sciences and Technology, the independent Faculty of Pharmacy was founded.

The development of the undergraduate study of pharmacy and higher professional study of laboratory biomedicine is based on connection between scientific research work and practical applications in all fields of pharmaceutical profession and science. Both study programmes have been modernized several times since their inception because staying abreast of modern developments and knowledge in pharmacy is essential for successful pedagogical, scientific and professional work.

The mission of the Faculty of Pharmacy:

1. Development, planning and implementation of pharmaceutical education, training of future professionals for conducting the most demanding scientific, development and professional work in the fields of pharmacy and clinical biochemistry.
2. Planning and implementation of scientific research work in the broader context of pharmacy, clinical biochemistry and natural sciences.
3. Care for professional activities in the fields of health and healthcare within the context of pharmaceutical activity, carrying out professional and development tasks for the needs of the pharmaceutical industry and government institutions, and promotion of the pharmaceutical profession in the Republic of Slovenia and abroad.

The primary objective of the Faculty of Pharmacy is to develop scientifically and professionally-qualified, high-quality graduates familiar with ethical principles, who independently carry out demanding tasks in pharmacies, pharmaceutical industry, hospital pharmacies, clinical biochemical laboratories, in laboratories for control and analyses, research institutions, educational organizations, state bodies and wherever the work and presence of a pharmacist is required to increase health safety.

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Lek d.d., a Sandoz company

Lek, a Sandoz company**,** is one of the pillars of leading world generics company Sandoz. It operates as a global development center for products and technologies, as a global manufacturing center for active pharmaceutical ingredients and medicines, as a competence center for the development of vertically integrated products, as a Sandoz competence center in the field of development and manufacturing of biopharmaceutical products and as a supply center for the markets of CEE, SEE and CIS, sales Slovenia and sales services for Sandoz's global markets. Lek d.d. has more than 2.400 employees in Slovenia.

In the R&D Division, active ingredients and pharmaceutical products are developed according to the latest breakthroughs in medicine and pharmacy. The research work is crucial in developing safe, effective and high quality drugs. Innovations in organic synthesis and pharmaceutical technology are essential in producing patient friendly drugs.

In Lek, the most advanced tools available to develop active ingredients - ranging from organic synthesis, classical biotechnology and genetic engineering and aided by the appropriate information technology and computerized modeling.

Pharmaceutical technology combines knowledge and understanding of active ingredients, auxiliary substances, technological processes and equipment and plays a key role in developing new drugs. Drug development is multidisciplinary experimental work, involving a pharmaceutical technologist who selects the optimum formula, production technology and the packaging for a specific pharmaceutical form. This is crucial for developing safe, effective and high quality drugs.

Safety and efficacy are the essential characteristics for any pharmaceutical product. Even pharmaceutical products under development, must undergo extensive testing of the physical and chemical properties of their ingredients and all other auxiliary substances used in their preparation. Lek uses the latest analytical methods to test and develop patient friendly drugs.
Products under development are evaluated by investigating their stability and by testing them in preclinical, pharmacokinetic and clinical studies. Only by complying with the strict regulations of the pharmaceutical industry (Good Practices, meeting pharmacopoeial requirements, legal provisions, as well as recommendations and guidelines of regulatory agencies), is Lek able to produce safe and effective medicines.

Phytochem Anal 2007; 18: 123-132.

A new method for the authentication of plant samples by analyzing fingerprint chromatograms

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Chemical analysis by high-performance liquid chromatography or capillary electrophoresis of plant pulverized samples, juices or extracts is an excellent method for the authentication of medicinal plant species and their products, particularly when morphological authentication is not possible. In the conventional procedure, chromatograms are integrated and the heights or areas of several peaks are used in a supervised pattern recognition method to confirm the authenticity of the product. We propose a new section approach in analysing chromatograms, where chromatograms are split into sections, which are described by four variables (number of peaks in the section, average retention time of peaks in the section, total area of peaks in the section and average area of peaks in the section), and these variables are then used in statistical analysis. The method is especially useful when the peaks on the chromatogram are not well separated and it is not easy to link individual peaks on one chromatogram with corresponding peaks on other chromatograms. In comparison with the standard procedure, our approach in analyzing chromatographic data of willow-herb (Epilobium and Chamaenerion spp.) extracts was more objective, gave better results and was also easier to perform.

Acta Chim Slov 2008; 55: 233-235.

Affinity ranking of phage-displayed peptides: enzyme-linked immunosorbent assay versus surface plasmon resonance

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Peptides with particular affinity and specificity for variety of targets are selected through panning procedure from random peptide phage display libraries. Efficiency and convenience of enzyme linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) for screening and evaluating peptide-displaying phage clones were compared using streptavidin as a model protein target.

J Pharm Biomed Anal 2007; 43: 243-249.

Anion-exchange chromatography using short monolithic columns as a complementary technique for human serum albumin depletion prior to human plasma proteome analysis

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In order to enable the detection of low abundance proteins from human plasma, it is necessary to remove high abundance proteins. Among them, human serum albumin and immunoglobulin G represent more than 75% of all such proteins. In this paper, the characterization of short monolithic columns was performed followed by the optimization of a multidimensional approach, known as conjoint liquid chromatography, to deplete human serum albumin and immunoglobulin G from a human plasma sample. Two different chromatographic modes were used: ion-exchange chromatography and affinity chromatography. A monolithic stationary phase (convective interaction media disk) bearing strong anion-exchange groups and another immobilized with protein G were placed in series into one housing. The optimal binding conditions were found that removed a majority of human serum albumin and immunoglobulin G from the human plasma sample. This method was compared to the depletion using a combination of pseudo-affinity and affinity columns. The results of the human serum albumin and immunoglobulin G depletion were confirmed by 2D electrophoresis. It has been shown that anion-exchange and affinity chromatography using convective interaction media monolithic columns can represent an efficient complementary technique for human serum albumin and immunoglobulin G removal from human plasma.

J Chem Inf Model 2007; 47: 737 -743.

Chemometric approach in quantification of structural identity/similarity of proteins in biopharmaceuticals

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We present a chemometrics study in which we show the identity or degree of similarity of 3D protein structures of various G-CSF (Granulocyte Colony-Stimulating Factor) isolates. The G-CSF isolates share the same amino acid sequence, but the preparation was carried out by somehow diverse technologies. The comparison of 3D structures was made on the basis of 2D NMR NOESY (Nuclear Overhauser Enhancement Spectroscopy) spectra of proteins. In searching for the most appropriate criteria to determine the identity or degree of similarity of selected spectral regions of different isolates, two methods for quantitative evaluation of identity/similarity were used. The first method compares all peaks in the two investigated protein spectral regions; the extent of peaks that overlap is determined. The second method includes spectral invariants originating from graph theory. The criteria of identity/similarity were calculated from graphs, derived from a collection of up to 200 peaks of investigated 2D NMR spectral region. The peaks were linked into a graph according to the sequential nearest neighborhoods. According to the first method all peaks were relevant, considering that spectral noise was previously removed; the largest similarity was found between the protein of a commercially available G-CSF drug and one of the three new isolates produced in the laboratory. The second method indicated that the pairwise similarity of the three new isolates is larger than the similarity of any of the new isolates with the commercially available drug. This is an expected result taking into account that the new isolates are produced by the same technology, while the commercial product has additives for long-term storage that could not be completely compensated. The proposed measure of similarity may help the developers of biosimilar products to optimize the controllable parameters of the production technology and eventually to argue the identity of the new isolate in comparison with the originator commercial product.

Anal Chim Acta 2008; 620: 150-161.

Comparative study of robustness between micellar electrokinetic capillary chromatography and high-performance liquid chromatography using one-variable-at-a-time and a new multi-variable-at-a-time approach

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Micellar electrokinetic capillary chromatography and reverse-phase liquid chromatography methods were developed in order to perform robustness testing to determine the caffeine content in beverages. Both methods were fully validated and two different robustness approaches were applied. One-variable-at-a-time (OVAT) approach at eleven levels (0; ± 1; ± 2; ± 3; ± 4; ± 5 units) was carried out and compared with multi-variable-at-a-time (MVAT) approach at three levels (± 1 unit per investigated parameter). Four analysts in two laboratories on two capillary electrophoresis and two RPLC equipments have tested the samples. Robustness was statistically analyzed using peak area, migration or retention time, symmetry, and resolution of caffeine and sulfacetamide as internal standard, and presented as RSD values. The RPLC method was found to be more sensitive than the MEKC method. Both methods showed acceptable robustness level for OVAT approach, whilst MEKC method was more robust when the determination of real samples coming from different beverages was based on the MVAT approach.

J Pharm Biomed Anal 2008; 46: 609–614.

Comparison of capillary electrophoresis and high performance liquid chromatography for determination of flavonoids in Achillea millefolium

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Flavonoids represent an important bioactive component in Achillea millefolium. The comparison of the most commonly used analytical methods for the identification and quantification of flavonoids, capillary electrophoresis (CE) and high performance liquid chromatography (HPLC), is presented. The methods were optimized and validated. Using a 20mM borate buffer with 30% (v/v) of methanol (pH 9.3) in the CE analysis and a gradient elution with water–acetonitrile mobile phase in the HPLC analysis, sufficient separation of the analytes was achieved. A relatively high injection volume in the CE analysis (30 mbar 30 s) enabled low limit of detection (LOD) (0.3–0.7 mg/L). Repeatability of both methods was acceptable (relative standard deviation of peak area were <6%). Additionally, the amount of flavonoids in a real sample of the dried herbal drug was determined.

J Sci Food Agric 2008; 88: 1576-1581.

Densitometric Determination of Zinc Bacitracin and Nystatin in Animal Feed

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BACKGROUND: The European Union has forbidden the use of antibiotics as an additive in animal feed. Zn-bacitracin and nystatin were frequently used for their growth-promoting effects and for feed conversion in poultry, pigs and cattle. An HPTLC method has been developed for separating Zn-bacitracin and nystatin in the mixture, for routine quality control.

RESULTS: The separation was obtained using RP-18 F_{254S} coated HPTLC plates with methanol/acetonitrile (equal volumes) : toluene : KH_2PO_4/KOH (buffer, pH 6.8) = 57 : 3 : 40 (v/v/v), adjusted with HCl to pH 8.2, as a mobile phase. The densitograms were monitored at 192, 215 and 305 nm and both antibiotics were assayed at 215 nm. The method was shown to be specific, accurate (recoveries were 98.7 0.5 % and 104.8 0.7 % for Zn-bacitracin and nystatin, respectively), linear over the tested range (correlation coefficients 0.9982 and 0.9884), and precise (intermediate precision RSD below 2.2 % for both analytes) with efficient separation (R_S = 3.5).

CONCLUSION: The method was applied for determining Zn-bacitracin and nystatin, as additives in spiked matrices of commercial animal feedstuffs. According to LOD values for each antibiotic, the minimum detectable amount in feed is 4.5 and 5.5 ppm of Zn-BC and NYS, respectively.

J Chromatogr A 2009; 1216: 2689-2694.

Depletion of high-abundance proteins from human plasma using a combination of an affinity and pseudo-affinity column

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Human serum albumin (HSA) and immunoglobulin G (IgG) represent over 75% of all proteins present in human plasma. These high-abundance proteins prevent the detection of low-abundance proteins which are potential markers for various diseases. The depletion of HSA and IgG is therefore essential for further proteome analysis. In this paper we describe the optimization of conditions for selective depletion of HSA and IgG using affinity and pseudo-affinity chromatography. A BIA Separations CIM (convective interaction media) Protein G disk was applied for the removal of IgG and the Mimetic Blue SA A6XL stationary phase for the removal of HSA. The binding and the elution buffer for CIM Protein G disk were chosen on the basis of the peak shape. The dynamic binding capacity was determined. It was shown to be dependent on the buffer system used and independent of the flow rate and of the concentration of IgG. Beside the binding capacity for the IgG standard, the binding capacity was also determined for IgG in human plasma. The Mimetic Blue SA A6XL column was characterized using human plasma. The selectivity of the depletion was dependent on the amount of human plasma that was loaded on the column. After the conditions on both supports had been optimized, the Mimetic Blue SA A6XL stationary phase was combined with the CIM Protein G disk in order to simultaneously deplete samples of human plasma. A centrifuge spin column that enables the removal of IgG and HSA from 20 μL of human plasma was designed. The results of the depletion were examined using sodium dodecyl sulfate polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis.

J Chromatogr Sci 2008; 46: 137-143.

Determination of Caffeine and Associated Compounds in Food, Beverages, Natural Products, Pharmaceuticals and Cosmetics by Micellar Electrokinetic Capillary Chromatography

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A method for quantifying caffeine, theobromine, theophylline, paracetamol, propyphenazone, acetylsalicylic acid, salicylic acid and codeine phosphate in corresponding real samples of food, beverages, natural products, pharmaceuticals and cosmetic preparations by micellar electrokinetic capillary chromatography (MEKC) is described. The separation was carried out at 25°C and 25 kV, using a 20 mM phosphate buffer (pH 9.0), 80 mM sodium dodecyl sulfate (SDS) and 7.5 % (v/v) acetonitrile. UV detection was at 210 nm. The method was shown to be specific, accurate (recoveries over the range 98.9 - 101.2 %), linear over the tested range (correlation coefficients ≥ 0.9993) and precise (RSD below 2.1 %). The method has been applied for quantitative analysis of these compounds in different food, beverages, natural products, pharmaceutical and cosmetic products.

Anal Bioanal Chem 2007; 387: 695-701.

Determination of doxycycline in pharmaceuticals and human urine by micellar electrokinetic capillary chromatography

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A micellar electrokinetic capillary chromatography (MEKC) was performed at 25C and 30 kV (under pressure of 15 mbar), using 30 mM borate buffer, containing 60 mM sodium dodecysulfate (SDS) and 5% (v/v) methanol, as background electrolyte, pH 9.0, for determination of doxycycline. UV detection was at 350 nm. The method was shown to be specific, accurate (recovery was 100.3 ± 1.0%), linear over the tested range (correlation coefficients 0.9995) and precise (RSD below 1.9%). The method was applied to determine doxycycline in tablets, capsules and human urine after oral application.

Electrophoresis 2007; 28: 965-969.

Determination of xanthohumol in hops (Humulus lupulus L.) by nonaqueous cappillary electrophoresis

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Xanthohumol (XN) is a prenylated chalcone with antimutagenic and anticancer activity from hops. A nonaqueous reverse polarity capillary electrophoretic method for the determination of XN in hop extract was developed and validated. The optimal parameters were a 64.5 cm long fused-silica capillary with 50 mu m id at 25 degrees C; 30 kV negative voltage (anode at detector side of the capillary); nonaqueous buffer with 75 mM NaOH and 50 mM boric acid in methanol; hydrodynamical injection with 10 mbar for 40 s; and detection at 440 nm. XN, isoxanthohumol (IX), colupulone, adlupulone, and n-lupulone were well resolved on the electropherogram. The LOD for XN was 0.05 mg/L and RSD for peak area was below 3%. The amount of XN in different samples of hop pellets varied from 0.14 to 0.42%.

Food Chem 2009; 1: 120-124.

Identification of buckwheat (Fagopyrum esculentum Moench) aroma compounds with GC-MS

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Buckwheat has a strong characteristic aroma, but its phytochemical background has not yet been fully elucidated. The aims of this study were identification and quantification of individual compounds responsible for the buckwheat aroma. Volatiles from a freshly ground buckwheat flour were extracted by different methods direct extraction with petroleum ether, pentane or methanol, distillation with Clevenger apparatus and a headspace solid-phase microextraction method. The extracts were analysed by GC-MS with electron ionisation. Compounds were identified by MS and by comparison of retention times with reference compounds. Direct extraction with methanol and distillation proved to be very efficient. In these extracts twenty-five and thirty-five compounds were identified, respectively. The first extract contained more hydrophilic compounds and the latter more volatile compounds. Most of the compounds were quantified and their odour activity value (OAV) calculated. Only two compounds (salicylaldehyde and phenylacetaldehyde) were found in both extracts. The compounds with the highest contribution to the buckwheat aroma were 2,5-dimethyl-4-hydroxy-3(2H)-furanone, (E,E)-2,4-decadienal, phenylacetaldehyde, 2-methoxy-4-vinylphenol, (E)-2-nonenal, decanal, hexanal and salicylaldehyde (2-hydroxybenzaldehyde).

Chem Biodivers 2008; 5: 310-317.

Identification of herbarium whole-leaf samples of Epilobium species by ATR-IRspectroscopy

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A simple, high-accuracy FT-IR method based on attenuated total reflection (ATR) was developed for the rapid determination of leaf samples of Epilobium species. The method is superior to other analytical techniques, since there is no need of laborious sample preparation such as grinding or extraction and solvent removal. A total of 70 herbarium specimens, belonging to all 13 Epilobium and to 2 Chamerion species growing in Slovenia, were analyzed. With the 100 most-informative wavenumbers in the range 700-1800 cm(-1), we obtained over 90% accuracy of species identification, with discriminant multivariate statistical analysis on the measurements made on whole dried leaves.

Chem Biol Drug Des 2007; 69: 124-131.

Improved acylation method enables efficient delivery of functional palmitoylated cystatin into epithelial cells

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The effective delivery of therapeutic proteins to the site of action is of great importance in achieving an effective therapy. Due to hydrophilicity, proteins are generally poorly transported across biological membranes. Chemical acylation represents one of the basic methods for improving their membrane permeability. A novel method for acylation is presented, based on the formation of palmitoylchloride dispersion in aqueous acetonitrile solution, using chicken cystatin as a model protein. We examined the effects of palmitoylchloride/cystatin molar ratio, reaction pH and introduction of successive palmitoylation cycles on the protein modification degree. The reaction products were analysed by capillary electrophoresis and SDS-PAGE, and the in vitro inhibitory activity was determined by N-benzoyl-D,L-arginine-beta-naphthylamide assay. Using cell culture-based assays, we examined the transport properties of unmodified and palmitoylated cystatin, its efficiency to inhibit intracellular enzymes, and its cytotoxicity. We demonstrated that palmitoylated cystatin rapidly internalized into the cell and caused a complete loss of cathepsin B activity. In contrast, the unmodified control cystatin was unable to inhibit the intracellular enzymes. These results strongly suggest protein palmitoylation to be a very effective strategy for improving cell internalization.

J Food Drug Anal 2008; 16: 18-25.

Optimal Conditions for Extraction and Simultaneous Determination of Sulfamethoxazole and Trimethoprim in Pharmaceuticals by Micellar Electrokinetic Capillary Chromatography

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An micellar electrokinetic capillary chromatography was performed at 25C and 30 kV (under pressure of 15 mbar), with 30 mM borate buffer (pH 9.0), 60 mM sodium dodecysulfate, and 10% (v/v) ethanol as background electrolyte for the determination of sulfamethoxazole and trimethoprim. UV detection was at 205 nm. Recoveries were optimal and acceptable after extraction with ethanol – deionized water (1:1; v/v) for both investigated compounds from laboratory mixtures of standards. The method was shown to be specific, accurate (recoveries were 99.9 ± 0.4 for sulfamethoxazole and 99.8 ± 0.3 for trimethoprim), linear over the tested ranges (correlation coefficients ≥ 0.9990) and precise (RSD below 0.6%). The method was applied to determine sulfamethoxazole and trimethoprim in tablets, powder for cutaneous use and solution for infusion.

Anal Chim Acta 2007; 594: 119-127.

Precision of micellar electrokinetic capillary chromatography in the determination of seven antibiotics in pharmaceuticals and feedstuffs

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Validation of analytical procedures is important for their efficient and reliable application. The ICH (International Conference on Harmonisation), FDA (Food and Drug Administration) and pharmacopoeia guidelines achieved a great deal in harmonising the definitions of the required validation characteristics. It is well known that poor reproducibility limits the practical implementation of capillary electrophoresis (CE). A precision study on four different MEKC methods were performed with eleven samples, containing seven antibiotics, by two analysts, in few days, on two capillary electrophoresis instruments. Five pharmaceutical preparations and three animal feeds were used. Precision was statistically analysed using migration time, peak area and height of each compound, as well as electroosmotic flow (EOF). In 22 of 27 cases, the reproducibility of peak area, peak height and migration time was good (< 5 %). In most cases the reproducibility of peak area was much better than the reproducibility of peak height. The worst reproducibility that we observed was 12.7 % for peak height and 7.6 % for peak area.

Drug Devel Ind Pharm 2008; 34: 547-557.

Preservative efficacy screening of pharmaceutical formulations using ATP bioluminescence

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The preservative challenge test is a method used to determine the efficacy of a preservation system in a pharmaceutical or cosmetic formulation. However, such testing is a labor-intensive, repetitive task often requiring days before results can be generated. Several alternatives to traditional colony-count techniques have been developed. A study using pure suspensions of Staphylococcus aureus, Pseudomonos aeruginosa, Escherichia coli, Bacillus subtilis, Candida albicans, and Aspergillus niger showed that the accuracy, repeatability, and linearity of the Pallchek(TM) luminometer ATP bioluminescence (ATP-B) system was equivalent to the traditional colony-count method. In any case, the method proved sensitive enough to follow the effect of preservatives on a number of test microorganisms, indicating the applicability of the ATP-B method for preservative screening studies in various pharmaceutical formulations.

J Chromatogr B Analyt Technol Biomed Life Sci 2007; 850: 531-537.

Sensitive electrochemical detection method for alpha-acids, beta-acids and xanthohumol in hops (Humulus lupulus L.)

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A new HPLC method with coulometric detection for the quantification of xanthohumol, alpha-acids and beta-acids in hops was developed. The separation of compounds was accomplished with a C18 column and isocratic elution with methanol: 50 mM potassium phosphate: ortho-phosphoric acid=80:20:0.25 (v/v/v). The method was validated and UV and electrochemical detectors (ECD) were compared. The HPLC method with ECD was precise, accurate and very sensitive for detection of xanthohumol and alpha- and beta-acids. The detection limits of analytes were at least 8.8 to 24 times lower with ECD than those of the UV detector. The ECD method was successfully applied for quantification of studied compounds in hop pellets. The concentrations of all compounds obtained with ECD and UV were found to be equivalent. This is the first study demonstrating a very sensitive and validated method for the quantification of xanthohumol, alpha- or beta-acids in hop samples with the use of the electrochemical detector.

J Chromatogr Sci 2008; 46: 144-149.

Simultaneous HPLC Determination of Caffeine, Theobromine and Theophylline in Food, Drinks and Herbal Products

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A rapid and selective high performance liquid chromatographic (HPLC) method has been developed for the separation and determination of caffeine, theobromine and theophylline. The chromatography was performed on a Zorbax Eclipse XDB-C8 column (4.6 mm x 150 mm, i.d., 5 μm particle size) at 25°C, with a mobile phase of water/THF (0.1 % THF in water, pH 8) – acetonitrile (90:10, v/v). The flow rate was 0.8 mL/min, and detection by UV at 273 nm. This method permits the simultaneous determination of caffeine, theobromine and theophylline in food, drinks and herbal products with detection limits of 0.07 – 0.2 mg/L and recoveries of 100.20 – 100.42%. Correlation coefficients, for the calibration curves in the linear range of 0.2 – 100 mg/L, were greater than 0.9999 for all compounds. The within- and between-day precision was determined for both retention times and peak area. The data suggested that the proposed HPLC method could be used for a routine quality control of food, drinks and herbal products.

Electrophoresis 2008; 29: 4431-4438.

Solid-phase extraction and large-volume sample stacking in MEKC for determination of doxycycline in biological fluids: Comparison of direct injection to SPE-MEKC

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A novel and simple method has been developed for the determination of doxycycline in biological fluids. The method is based on SPE, LVSS and MEKC with UV-DAD detection. Six SPE cartridges have been used in investigation for sample clear up and pre-concentration (Supelco® LC-8, LC-18, LC-SCX, and LC-WCX, as well as Strata™-X and X-C). Doxycycline was determined on a 56 cm (effective length 50 cm) x 50 m id fused silica capillary. The BGE was 20 mM borate buffer, pH 9.3, containing 80 mM SDS and 7.5% (v/v) of methanol (30 s x 50 mbar), and the temperature and voltage were 25C and 30 kV, respectively. The analytical wavelength was set at 210 nm. Under optimised conditions it is possible to determine doxycycline in human serum, urine, semen, tears and saliva with recovery of 97.5% (RSD 2.5%). The method was shown to be sensitive (LOD is 1 μg/L), and precise (intra-day RSD 0.2 and 2.4%; inter- days 0.4 and 3.5% for migration time and peak area, respectively). Results for developed SPE-LVSS-MEKC were compared with LVSS-MEKC method with direct sample injection. The new LVSS-MEKC method is presented as a useful technique for rapid determination without extraction procedure of doxycycline in human urine and serum, using 80 mM of SDS, 10% (v/v) of methanol and 40 mM borate buffer (pH 9.3; 30 s x 50 mbar; 25C; 30 kV; 350 nm), but not for the other biological fluids, according to lower sensitivity of the method and because of the sample composition.

J Chromatogr Sci 2007; 45: 623-628.

Thermostability testing and degradation profiles of doxycycline in bulk, tablets and capsules by HPLC

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An HPLC method for the quantitation of doxycycline in bulk, tablets and capsules after storage at -20, 5, 25, 40, 50, 60 and 70ºC, has been developed and validated. The samples were eluted from a -Bondapak C_8 column (4.6 mm x 150 mm, i.d., 5 μm particle size) at 27°C, with a mobile phase of acetonitrile/water/THF (29.5 : 70 : 0.5, v/v/v), adjusted to pH 2.5 with 1.0 M HCl. The flow rate was 1.0 ml/min, and detection by UV at 350 nm. The stability of doxycycline in bulk and in pharmaceuticals was checked over 90 days. Doxycycline showed thermo-degradation after exposure to high temperature; tablets are more stable than capsules. The shelf lives ($t_{90\%}$) were determined to be 1.00, 2.84 and 5.26 years in bulk, capsules and tablets, respectively at 25°C. Metacycline and 6-epidoxycyline were identified as degradation products at high temperatures. Amounts of doxycycline, metacycline and 6-epidoxycycline in all samples were determined by HPLC and the results compared with those from micellar electrokinetic capillary chromatography. After 90 days metacycline and 6-epidoxycyline were almost equal in test samples from standard bulk form, tablets and capsules. It was 27.8 ± 0.3%, 13.7 ± 0.1% and 18.8 ± 0.2%, respectively.

Int J Pharm 2008; 356: 200-205.

The use of microcalorimetry and HPLC for the determination of degradation kinetics and thermodynamic parameters of Perindopril Erbumine in aqueous solutions

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Perindopril Erbumine (PER) is one of the newly used angiotensin-converting enzyme inhibitors (ACE inhibitors) and is used for the treatment of patients with hypertension and symptomatic heart failure. It has two main degradation pathways, i.e. the degradation by hydrolysis and the degradation by cyclization. An isothermal heat conduction microcalorimetry (MC) and high pressure liquid chromatography (HPLC) were used for the characterization of aqueous solutions of PER and its stability properties. The rates of heat evolved during degradation of perindopril were measured by MC as a function of temperature and pH and from these data rate constant and change in enthalpy of the reactions were determined. With the HPLC method the concentration of perindopril and its degradation products were measured as a function of time in aqueous solutions of different pH that were stored at different temperatures. We demonstrated that reactions of degradation of perindopril at observed conditions follow the first order kinetics. The Arrhenius equation for each pH was determined. At pH 6.8 only one degradation pathway is present, i.e. the degradation by hydrolysis. Degradation constants for this pathway calculated from MC data are in good agreement with those obtained from HPLC. MC as a non-specific technique was shown to be useful in studies of PER when one reaction was present in the sample and also when more chemical and physical processes were simultaneously running.

J Therm Anal Calorim 2008; 92: 791-794.

Use of isothermal microcalorimetry for prediction of oxidative stability of several amino acids

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Isothermal microcalorimetry has been applied as a method for predicting (in)stability of ascorbic acid and several amino acids that undergo oxidative degradation in aqueous media. The fast and simple method involved the addition of different amounts of hydrogen peroxide. The appearance of the heat flow curves gave a clear general indication of how stability was influenced. The accuracy of the microcalorimetric result was investigated by comparing it with an HPLC assay and a good agreement between the results of both methods was demonstrated. It was also established that susceptibility to oxidative degradation decreases in the following order: cysteine, methionine, ascorbic acid, tyrosine and tryptophan.

Int J Pharm 2007; 342: 145-151.

Use of microcalorimetry in determination of stability of enalapril maleate and enalapril maleate tablet formulations

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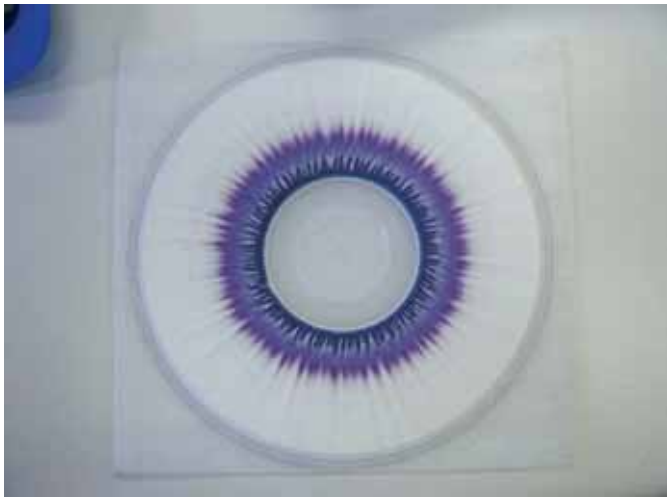
The stability properties of enalapril maleate (EM) and of different tablet formulations including EM were studied by isothermal microcalorimetry and by high performance liquid chromatography (HPLC). It was shown that water content of the sample and elevated temperature have a high impact on stability properties of the substance itself and of the formulations including this substance. The degradation is more extensive at higher water content and at elevated temperature. The type of the tablet formulation (5 or 20 mg EM tablet formulation) also has an impact: the 5 EM tablet formulation is the less stable one. The heat output of individual tablet formulations was used to evaluate the enthalpy changes and to calculate the difference in the amount of degraded EM between various samples. These results agreed satisfactorily with those obtained by HPLC. Isothermal microcalorimetry proved to be a fast and predictive method that could be used in preformulation studies to accelerate the pharmaceutical development and shorten the time before launching the product to the market.



J Bacteriol 2007; 189: 3987-3995.

Biochemical characterization and physiological properties of Escherichia coli UDP-Nacetylmuramate: L-alanyl-γ-D-glutamyl-meso-diaminopimelate ligase

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The UDP-N-acetylmuramate: L-alanyl-γ-D-glutamyl-meso-diaminopimelate ligase (murein peptide ligase [Mpl]) is known to be a recycling enzyme allowing reincorporation into peptidoglycan (murein) of the tripeptide L-alanyl-γ-D-glutamyl-meso-diaminopimelate released during the maturation and constant remodeling of this bacterial cell wall polymer that occur during cell growth and division. Mpl adds this peptide to UDP-N-acetylmuramic acid, thereby providing an economical additional source of UDP-MurNAc-tripeptide available for de novo peptidoglycan biosynthesis. The Mpl enzyme from Escherichia coli was purified to homogeneity as a His-tagged form, and its kinetic properties and parameters were determined. Mpl was found to accept tri-, tetra-, and pentapeptides as substrates in vitro with similar efficiencies, but it accepted the dipeptide L-Ala-D-Glu and L-Ala very poorly. Replacement of meso-diaminopimelic acid by L-Lys resulted in a significant decrease in the catalytic efficacy. The effects of disruption of the E. coli mpl gene and/or the ldcA gene encoding the LD-carboxypeptidase on peptidoglycan metabolism were investigated. The differences in the pools of UDP-MurNAc peptides and of free peptides between the wild-type and mutant strains demonstrated that the recycling activity of Mpl is not restricted to the tripeptide and that tetra- and pentapeptides are also directly reused by this process in vivo. The relatively broad substrate specificity of the Mpl ligase indicates that it is an interesting potential target for antibacterial compounds.

FEMS Microbiol Rev 2008; 32:168-207.

Cytoplasmic steps of peptidoglycan biosynthesis

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The biosynthesis of bacterial cell wall peptidoglycan is a complex process that involves enzyme reactions that take place in the cytoplasm (synthesis of the nucleotide precursors) and on the inner side (synthesis of lipid-linked intermediates) and outer side (polymerization reactions) of the cytoplasmic membrane. This review deals with the cytoplasmic steps of peptidoglycan biosynthesis, which can be divided into four sets of reactions that lead to the syntheses of : i) UDP-*N*-acetylglucosamine from fructose 6-phosphate; ii) UDP-*N*-acetylmuramic acid from UDP-*N*-acetylglucosamine; iii) UDP-*N*-acetylmuramyl-pentapeptide from UDP-*N*-acetylmuramic acid; and iv) D-glutamic acid and dipeptide D-alanyl-D-alanine. Recent data concerning the different enzymes involved are presented. Moreover, special attention is given to: i) the chemical and enzymatic synthesis of the nucleotide precursor substrates that are not commercially available, and ii) the search for specific inhibitors that could act as antibacterial compounds.

Curr Pharm Des 2007; 13: 2283-2309.

Development of novel inhibitors targeting intracellular steps of peptidoglycan biosythesis

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The widespread emergence of pathogenic bacterial strains with resistance to antibiotics is becoming a serious threat to public health. Continuous development of novel antibacterials therefore remains one of the biggest challenges to science and unmet needs in the clinics. The biosynthetic pathway of bacterial peptidoglycan, an essential building block of cell walls, has been well studied and appears to be a rich source of attractive enzyme targets for new antibacterials. We have therefore reviewed the intracellular part of peptidoglycan biosynthesis, including the enzymes GlmS, GlmM, GlmU for formation of UDP-GlcNAc, subsequent pentapeptide synthesis by MurAMurF, and its connection to lipid carrier by MraY and MurG. Naturally occurring inhibitors and the development of low-molecular weight inhibitors of the intracellular part of peptidoglycan synthesis are presented.

Curr Med Chem 2007; 14: 2033-2047.

Discovery and development of ATPase inhibitors of DNA gyrase as antibacterial agents

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DNA gyrase is an attractive and well established target for the development of antibacterial agents. This bacterial enzyme, whose biological function is to control the topological state of DNA molecules, consists of two catalytic subunits; GyrA is responsible for DNA breakage and reunion, while the subunit GyrB contains the ATP-binding site. Coumarins and cyclothialidines are natural products that inhibit the ATPase activity of DNA gyrase by blocking the binding of ATP to subunit GyrB. The mechanism of action of these compounds was exhaustively characterized by biochemical methods and supported by protein crystallography. The abundance of crystallographic data on the N-terminal domain of GyrB in its complexes with various ligands has enabled the structure-based design of novel efficient chemotypes as inhibitors of the ATPase domain. This review summarizes the discovery of ATPase inhibitors of DNA gyrase B in the last decade and their development as potential antibacterial agents.

J Med. Chem 2008; 51: 7442-7448.

Discovery of new inhibitors of D-alanine:D-alanine ligase by structure-based virtual screening

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The terminal dipeptide, D-Ala-D-Ala, of the peptidoglycan precursor UDP-MurNAc-pentapetide is a crucial building block involved in peptidoglycan cross-linking. It is synthesized in the bacterial cytoplasm by the enzyme D-alanine:D-alanine ligase (Ddl). Structure-based virtual screening of the NCI Diversity Set of almost 2000 compounds was performed with a DdlB isoform from *Escherichia coli* using the computational tool AutoDock 4.0. The 130 best ranked compounds from this screen were tested in an *in vitro* assay for their inhibition of *E. coli* DdlB. Three compounds were identified that inhibit the enzyme with better than D-cycloserine. Two of these also have promising antibacterial activities against Gram-positive and Gram-negative bacteria.

J Med Chem 2008; 51: 7486-7494.

Novel naphthalene N-sulfonyl-D-glutamic acid derivatives as inhibitors of MurD, a key peptidoglycan biosynthesis enzyme

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MurD (UDP-*N*-acetylmuramoyl-L-alanine:D-glutamate ligase) is the second enzyme in the series of Mur ligases and it catalyses the addition of D-glutamic acid (D-Glu) to the cytoplasmic intermediate UDP-*N*-acetylmuramoyl-L-alanine (UMA). Due to the high binding affinity of D-Glu towards MurD, we synthesized and biochemically evaluated a series of *N*-substituted D-Glu derivatives as potential inhibitors of MurD from *E. coli*, which allowed us to explore the structure-activity relationships. The substituted naphthalene *N*-sulfonyl-D-Glu inhibitors, which were synthesized as potential transition-state analogues, displayed IC_{50} values ranging from 80 μM to 600 μM. In addition, the high-resolution crystal structures of MurD in complex with four novel inhibitors revealed details of the binding mode of the inhibitors within the active site of MurD.

J Mol Biol 2007; 370: 107-115.

Structural and functional characterization of enantiomeric glutamic acid derivatives as potential transition state analogue inhibitors of MurD ligase

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Mur ligases play an essential role in the intracellular biosynthesis of bacterial peptidoglycan, the main component of the bacterial cell wall, and represent attractive targets for the design of novel antibacterials. UDP-*N*-acetylmuramoyl-l-alanine:d-glutamate ligase (MurD) catalyses the addition of d-glutamic acid to the cytoplasmic intermediate UDP-*N*-acetylmuramoyl-l-alanine (UMA) and is the second in the series of Mur ligases. MurD ligase is highly stereospecific for its substrate, d-glutamic acid (d-Glu). Here, we report the high resolution crystal structures of MurD in complexes with two novel inhibitors designed to mimic the transition state of the reaction, which contain either the d-Glu or the l-Glu moiety. The binding modes of *N*-sulfonyl-d-Glu and *N*-sulfonyl-l-Glu derivatives were also characterised kinetically. The results of this study represent an excellent starting point for further development of novel inhibitors of this enzyme.

J Med Chem 2007; 50: 4113-4121.

4-Substituted Trinems as Broad Spectrum β-Lactamase Inhibitors: Structure-Based Design, Synthesis, and Biological Activity

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A wide variety of pathogens have acquired antimicrobial resistance as an inevitable evolutionary response to the extensive use of antibacterial agents. In particular, one of the most widely used antibiotic structural classes is the β-lactams, in which the most common and the most efficient mechanism of bacterial resistance is the synthesis of β-lactamases. Class C β-lactamase enzymes are primarily cephalosporinases, mostly chromosomally encoded, and are inducible by exposure to some β-lactam agents and resistant to inhibition by marketed β-lactamase inhibitors. In an ongoing effort to alleviate this problem a series of novel 4-substituted trinems was designed and synthesized. Significant *in vitro* inhibitory activity was measured against the bacterial β-lactamases of class C and additionally against class A. The lead compound LK-157 was shown to be a potent mechanism-based inactivator. Acylation of the active site Ser 64 of the class C enzyme β-lactamase was observed in the solved crystal structures of two inhibitors complexes to AmpC enzyme from *E. cloacae*. Structure-activity relationships in the series reveal the importance of the trinem scaffold for inhibitory activity and the interesting potential of the series for further development

Lancet Inf dis 2008: 8: 133-139.

Treatment of health-care-associated infections caused by Gram-negative bacteria: a consensus statement

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This consensus statement presents the conclusions of a group of academic and industrial experts who met in London in September, 2006, to consider the issues associated with the treatment of hospital infections caused by Gram-negative bacteria. The group discussed the severe clinical problems arising from the emergence of antibiotic resistance in these bacteria and the lack of new antibacterial agents to challenge the threat. The discovery of new drugs active against hospital-acquired Gram-negative bacteria is essential to prevent a future medical and social catastrophe. An important strategy to promote drug discovery will be the development of focused cooperations between academic institutions and small pharmaceutical companies.

Int J Pharm 2007; 335: 106-113.

A new amoxicillin/clavulanate therapeutic system: Preparation, in vitro and pharmacokinetic evaluation

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A new peroral amoxicillin/clavulanate therapeutic system composed of immediate release tablet and controlled release floating capsule was developed and evaluated by in vivo bioavailability study. Pharmacokinetic (PK) parameters for amoxicillin and clavulanic acid of the new therapeutic systems: AUCt, AUCi, (AUCt/AUCi), Cmax, Tmax, kel, T1/2 and additionally for amoxicillin T4 and T2 were calculated from the plasma levels. The study confirmed enhanced pharmacokinetic parameters of a newly developed therapeutic system containing 1500 mg of amoxicillin and 125 mg of clavulanic acid. Prolonged time over MIC of amoxicillin in relation to a regular immediate release amoxicillin/clavulanate formulation was confirmed

Pharm Biol 2007; 45: 700-706.

Antibacterial activity in higher fungi (mushrooms) and endophytic fungi from Slovenia

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Discovery and development of new antibiotics is still very important because of the constant appearance of drug-resistant pathogenic bacteria. The broth microdilution test was applied for screening of antibacterial activity in extracts of higher and endophytic fungi. Among tested extracts, three possessed significant antibacterial activity: extracts of mushrooms Amanita virosa (Fr.) Bertill. (Amanitaceae) and Cortinarius praestans Cordier (Cortinariaceae) against Pseudomonas aeruginosa and Staphylococcus aureus, respectively, and extract of endophytic fungus Trucatella hartigii (Tubeuf) Steyaert (Amphisphaeriaceae) against Enterococcus faecalis and S. aureus. The extract of Truncatella hartigii was further analyzed by one- and two-dimensional thin-layer chromatography, and the position of the active compound was determined on the chromatogram.

J Biomol Screen 2009: 14; 142-150.

Comparison of 3 Cytotoxicity Screening Assays and Their Application to the Selection of Novel Antibacterial Hits

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Cytotoxicity screening of new chemical entities in antibacterial drug discovery discerns between cytotoxic and antimicrobial activity, thus providing predictive evidence for selective toxicity. The objective of this study was to evaluate 3 cytotoxicity assays in identifying novel antibacterial hits with desired safety margins. The endpoints in assays comprised adenylate kinase (AK) release rate as an indicator of membrane rupture (Toxilight (TM)), intracellular adenosine triphosphate (CellTiter-Glo (TM)), and reduction of resazurin (CellTiter-Blue (TM)) both as indicators of cell metabolic activity. In the CellTiter-Glo (TM) and the CellTiter-Blue (TM) assays, 7 of 8 selected compounds showed cytotoxicity, whereas in the Toxilight. assay, 3 of 8 compounds significantly reduced cell viability in the ChoK1 and the JurkatE6.1 cell line. The CellTiter-Glo (TM) assay proved to be the most sensitive among the evaluated assays, and excellent Z' values were obtained in the 96-well plate (Z' > 0.83). The CellTiter-Glo (TM) assay was clearly superior to the CellTiter-Blue (TM) and the Toxilight. assay for the initial cytotoxicity screening. Moreover, the application of the CellTiter-Glo (TM) assay to determine mammalian cell toxicity versus the antibacterial effect ratio contributed to early identification of antibacterial hits with desired safety margins. The chemical structures of these novel antibacterial hits are disclosed herein.

Bioorg Med Chem Lett 2009; 19: 1376-1379.

Design and synthesis of new hydroxyethylamines as inhibitors of D-alanyl-D-lactate ligase (VanA) and D-alanyl-D-alanine ligase (DdlB)

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The Van enzymes are ATP-dependant ligases responsible for resistance to vancomycin in *Staphylococcus aureus* and *Enteroccoccus* species. The *de novo* molecular design programme SPROUT was used in conjunction with the X-ray crystal structure of *Enterococcus faecium* D-alanyl-D-lactate ligase (VanA) to design new putative inhibitors based on a hydroxyethylamine template. The two best ranked structures were selected and efficient syntheses developed. The inhibitory activities of these molecules were determined on *E. faecium* VanA, and due to structural similarity and a common reaction mechanism, also on D-Ala-D-Ala ligase (DdlB) from *Escherichia coli*. The phosphate group attached to the hydroxyl moiety of the hydroxyethylamine isostere within these systems is essential for their inhibitory activity against both VanA and DdlB.

Molecules 2008; 13: 11-30.

Design and Synthesis of Novel N-Benzylidenesulfonohydrazide Inhibitors of MurC and MurD as Potential Antibacterial Agents

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A series of novel *N*-benzylidenesulfonohydrazide compounds were designed and synthesized as inhibitors of UDP-*N*-acetylmuramic acid: *L*-alanine ligase (MurC) and UDP-*N*-acetylmuramoyl-*L*-alanine: *D*-glutamate ligase (MurD) from *E. coli*, involved in the biosynthesis of bacterial cell-walls. Some compounds possessed inhibitory activity against both enzymes with IC_{50} values as low as 30 μM. In addition, a new, one-pot synthesis of amidobenzaldehydes is reported.

Bioorg Med Chem Lett 2007; 17: 2047-2054.

Diazenedicarboxamides as inhibitors of D-alanine-D-alanine ligase (Ddl)

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D-Alanine-D-alanine ligase (Ddl) catalyzes the biosynthesis of an essential bacterial peptidoglycan precursor D-alanyl-D-alanine and it represents an important target for development of new antibacterial drugs. A series of semicarbazides, aminocarbonyldiazenecarboxylates, diazenedicarboxamides, and hydrazinedicarboxamides was synthesized and screened for inhibition of DdlB from E. coli. Compounds with good inhibitory activity were identified, enabling us to deduce initial structure–activity relationships. Thirteen diazenedicarboxamides are better inhibitors than D-cycloserine and some of them also possess antibacterial activity, which makes them a promising starting point for further development.

Pharmazie 2007; 62: 318-320.

Influence of metal cations on the solubility of fluoroquinolones

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Although a clinically relevant interaction between a fluoroquinolone and a metal cation was first described more than 20 years ago the biopharmaceutical mechanism of this interaction is still not understood. One of the obvious disagreements in the literature is about the effect of metal cations on the solubility of fluoroquinolones. Namely, metal cations are reported to increase the solubility of fluoroquinolones as well as to decrease it and thus cause the lowered bioavailability. Thus in this work the solubility of ciprofloxacin, norfloxacin and ofloxacin and the effect of metal cations on the solubility of these fluoroquinolones in aqueous media prepared by different buffers and at different pH values were reevaluated. The results clearly show that the metal cations either do not affect or they even increase the solubility of fluoroquinolones. Thus they surely do not influence the bioavailability of these drugs by decreasing their solubility. Additionally, possible explanations for the contradictory results reported in the literature are given.

Antimicrob Agents Chemother 2009; 53: 505-511.

In Vitro Activity of LK-157, a Novel Tricyclic Carbapenem as Broad-Spectrum β-Lactamase Inhibitor

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LK-157 is a novel tricyclic carbapenem with potent activity against class A and class C β-lactamases. When tested against the purified TEM-1 and SHV-1 enzymes, LK-157 exhibited 50% inhibitory concentrations (IC50s) in the ranges of the clavulanic acid and tazobactam IC50s (55 nM and 151 nM, respectively). Moreover, LK-157 significantly inhibited AmpC β-lactamase (IC50, 62 nM), as LK-157 was >2,000-fold more potent than clavulanic acid and approximately 28-fold more active than tazobactam. The in vitro activities of LK-157 in combination with amoxicillin, piperacillin, ceftazidime, cefotaxime, ceftriaxone, cefepime, cefpirome, and aztreonam against an array of Ambler class A (TEM-, SHV-, CTX-M-, KPC-, PER-, BRO-, and PC-type)- and class C-producing bacterial strains derived from clinical settings were evaluated in synergism experiments and compared with those of clavulanic acid, tazobactam, and sulbactam. In vitro MICs against ESBL-producing strains (except CTX-M-containing strains) were reduced 2- to >256-fold, and those against AmpC-producing strains were reduced even up to >32-fold. The lowest MICs (0.025 to 1.6 g/ml) were observed for the combination of cefepime and cefpirome with a constant LK-157 concentration of 4 g/ml, thus raising an interest for further development. LK-157 proved to be a potent β-lactamase inhibitor, combining activity against class A and class C β-lactamases, which is an absolute necessity for use in the clinical setting due to the worldwide increasing prevalence of bacterial strains resistant to β-lactam antibiotics.

Chem-biol Interact 2009; 178: 310-316.

Novel inhibitors of ß-ketoacyl-ACP reductase from Escherichia coli

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Bacterial β-ketoacyl-[acyl carrier protein] (β-ketoacyl-ACP) reductase (FabG) is a highly conserved and ubiquitously expressed enzyme of the fatty-acid biosynthetic pathway of prokaryotic organisms that catalyzes NADPH-dependent reduction of β-ketoacyl-ACP intermediates. Therefore, FabG represents an appealing target for the development of new antimicrobial agents. A number of trans-cinnamic acid derivatives were designed and screened for inhibitory activities against FabG from Escherichia coli. These inhibited FabG enzymatic activity with IC50 values in the M range, and were used as templates for the subsequent diversification of the chemotype. Introduction of an electron-withdrawing 4-cyano group to the phenol substituent showed improved inhibition over the non-substituted compound. The benzo-[1,3]-dioxol moiety also appeared to be essential for inhibitory activity of trans-cinnamic acid derivatives against FabG from E. coli. To explain the possible binding position, the best inhibitor from the present study was docked in the active site of FabG. The results for the best scoring conformers chosen by the docking programme revealed that cinnamic acid derivatives can be accommodated in the substrate-binding region of the active site, above the nicotinamide moiety of the NADPH cofactor. Additionally, a phage-displayed library of random linear 15-mer peptides was screened against FabG, to identify ligands with the common PPLTXY motif.

J Basic Microbiol 2008; 48: 202-206.

Peptide inhibitors of MurD and MurE, essential enzymes of bacterial cell wall biosynthesis

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Continuous development of antibacterial compounds with novel modes of action (accompanied by rationalization of chemotherapeutic prescription) is the best way to address the growing problem of antibiotic-resistant infections. Numerous clinically important antibiotics interfere with peptidoglycan cell wall biosynthesis making this unique metabolic pathway a well validated target for antimicrobials. While nearly all of these antibiotics inhibit late stages of murein synthesis occurring on the extracellular side of plasma membrane, initial cytoplasmic steps have not been extensively exploited as drug targets. We performed affinity selection of peptides from phage-displayed libraries against two essential bacterial enzymes MurD and MurE involved in the cytoplasmic synthesis of peptidoglycan monomer. Selected peptides were found to inhibit respective target enzymes in an in vitro assay with IC50 values of 140 mu M to 1.5 mM. These peptides represent starting point for design of peptidomimetic lead compounds with the ultimate objective of small molecule chemotherapeutic development.

Appl Environ Microbiol 2007; 73: 1029-1032.

Prevalence of ColE1-Like Plasmids and Colicin K Production among Uropathogenic Escherichia coli Strains and Quantification of Inhibitory Activity of Colicin K

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Colicin K exhibited pronounced inhibitory activity against uropathogenic *Escherichia coli* (UPEC) strains. Low prevalence of colicin K production and a relatively high prevalence of ColE1-like plasmids were determined among 215 UPEC strains from Slovenia. Sequencing of the colicin K-encoding pColK-K235 revealed a mosaic structure and the presence of the insertion sequence IS*2*.

ChemMedChem 2008; 9: 1362-1370.

Synthesis and biological evaluation of N-acylhydrazones as inhibitors of MurC and MurD ligases

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The Mur ligases have an essential role in the intracellular biosynthesis of bacterial peptidoglycan, and they represent attractive targets for the design of novel antibacterials. A series of compounds with an N-acylhydrazone scaffold were synthesized and screened for inhibition of the MurC and MurD enzymes from Escherichia coli. Compounds with micromolar inhibitory activities against both MurC and MurD were identified, and some of them also showed antibacterial activity.

Bioorg Med Chem Lett 2009 ; 19 : 153-157.

Synthesis and Biological Evaluation of New Glutamic Acid Based Inhibitors of MurD Ligase

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Mur ligases catalyze the biosynthesis of an essential bacterial peptidoglycan precursor UDP-MurNAc-pentapeptide and represent attractive targets for development of novel antibacterial agents. Here we report the synthesis of a series of 2,4-diaminoquinazolines, quinazoline-2,4(1*H*,3*H*)-diones, 5-benzylidenerhodanines and 5-benzylidenethiazolidine-2,4-diones and their inhibitory activities against MurD from *Escherichia coli*. Compounds **(*R*)-27** and **(*S*)-27** showed inhibitory activity against MurD with IC_{50} values of 174 μM and 206 μM, respectively, which makes them a promising starting point for further optimization.

Proteins 2007; 68: 243-254.

Targeted molecular dynamics simulation studies of binding and conformational changes in E. coli MurD

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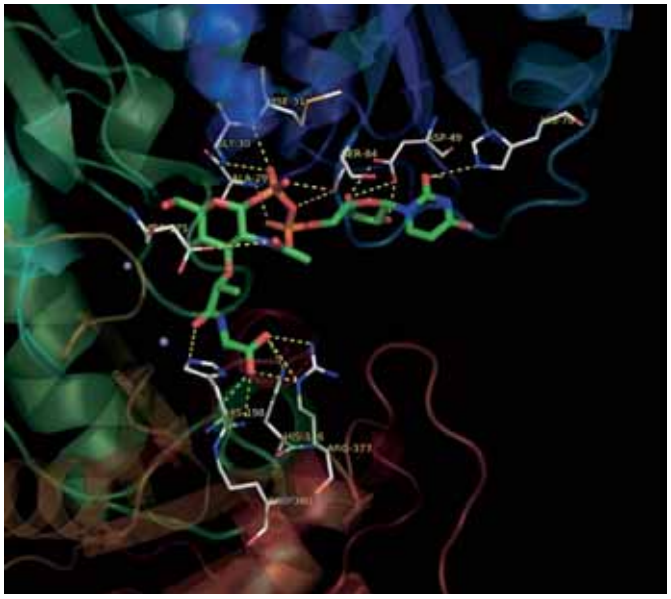
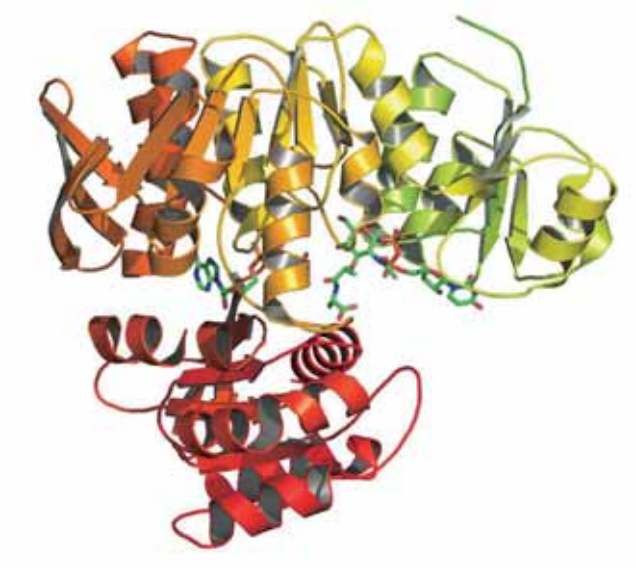
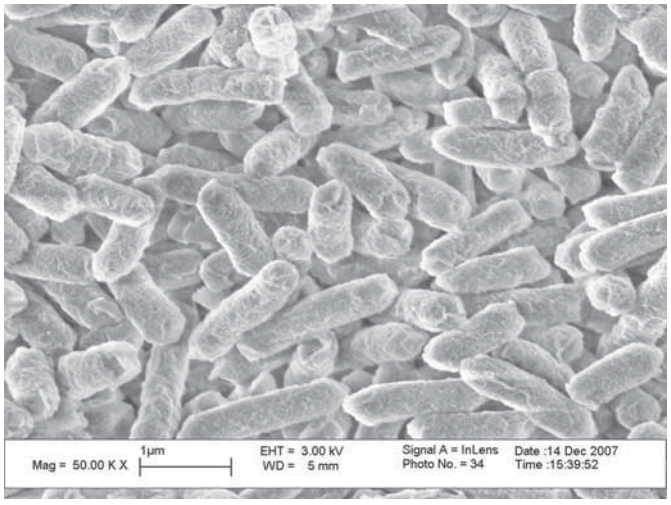
Enzymes involved in the biosynthesis of bacterial peptidoglycan, an essential cell wall polymer unique to prokaryotic cells, represent a highly interesting target for antibacterial drug design. Structural studies of E. coli MurD, a three-domain ATP hydrolysis driven muramyl ligase revealed two inactive open conformations of the enzyme with a distinct C-terminal domain position. It was hypothesized that the rigid body rotation of this domain brings the enzyme to its closed active conformation, a structure, which was also determined experimentally. Targeted molecular dynamics 1 ns-length simulations were performed in order to examine the substrate binding process and gain insight into structural changes in the enzyme that occur during the conformational transitions into the active conformation. The key interactions essential for the conformational transitions and substrate binding were identified. The results of such studies provide an important step toward more powerful exploitation of experimental protein structures in structure-based inhibitor design.



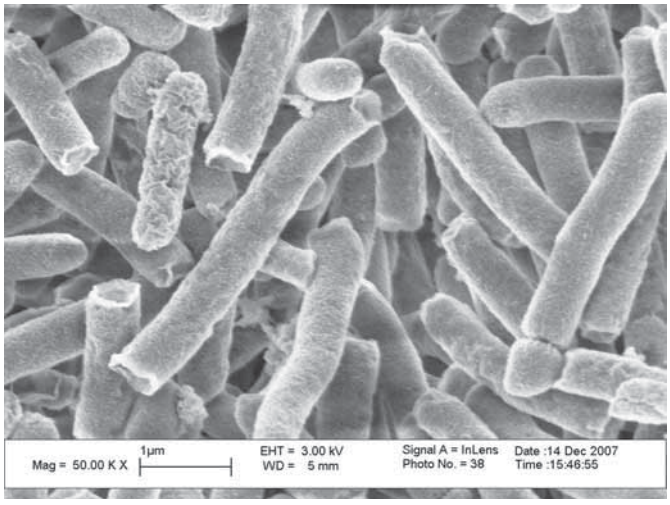
Active site of ligase MurC with the reaction product.



Ligase MurD , the substrate of the reaction and ATP.



E. coli (50000 magnified) Courtesy of Prof. Dr. Julianne Bostock, University of Leedst



E. coli with inhibitor of cell-wall biosynthesis (50000 magnified) Courtesy of Prof. Dr. Julianne Bostock, University of Leeds

Eur J Pharmacol 2009; 602:15-22.

Azaphenylalanine-based serine protease inhibitors induce caspase-mediated apoptosis

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Molecules regulating cell death constitute prominent therapeutic targets. The pro-apoptotic role of serine protease inhibitors prompted us to search for novel modulators of this process. We have tested some recently synthesized antithrombotic compounds for their potential to induce apoptotic cell death. Cell based analyses revealed that inhibitors built on the azaphenylalanine scaffold are, for B-cell lymphoma cells, severely cytotoxic, while other compounds tested were moderate or non-cytotoxic. These inhibitors induced the time and concentration dependent biochemical and morphological characteristics of apoptosis, such as DEVDase activation, loss of mitochondrial membrane potential, nuclear degradation and genomic DNA fragmentation. Most of the inhibitors proved to be selective for thrombin, with inhibition constants (K_i) in the nanomolar range. However, they could also inhibit at least one additional serine protease (trypsin, chymotrypsin and/or coagulation factor X) with K_i values in the nanomolar or low micromolar range. These serine protease inhibitors constitute novel apoptosis inducing compounds in B-cell lymphoma cells.

Proc Natl Acad Sci 2008; 105: 14808-14813.

Directed evolution of a G protein-coupled receptor for expression, stability, and binding selectivity

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We outline a powerful method for the directed evolution of integral membrane proteins in the inner membrane of *Escherichia coli*. For a mammalian G protein-coupled receptor, we arrived at a sequence with an order-of-magnitude increase in functional expression that still retains the biochemical properties of wild type. This mutant also shows enhanced heterologous expression in eukaryotes (12-fold in *Pichia pastoris* and 3-fold in HEK293T cells) and greater stability when solubilized and purified, indicating that the biophysical properties of the protein had been under the pressure of selection. These improvements arise from multiple small contributions, which would be difficult to assemble by rational design. In a second screen, we rapidly pinpointed a single amino acid substitution in wild type that abolishes antagonist binding while retaining agonist-binding affinity. These approaches may alleviate existing bottlenecks in structural studies of these targets by providing sufficient quantities of stable variants in defined conformational states.

Histochem Cell Biol 2009; 131: 129-139.

Rapid differentiation of superficial urothelial cells after chitosan induced desquamation

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Superficial cell desquamation followed by differentiation of newly exposed superficial cells induces regeneration of the urinary bladder epithelium, urothelium. In the present work, chitosan was evaluated as a new inducer of urothelial cell desquamation, in order to study the regeneration of mouse urothelial cells in vivo. Intravesical application of chitosan dispersion caused complete removal of only the superficial layer of cells within 20 min of treatment. Differentiation of the new superficial layer was followed by the appearance and distribution of three urothelial differentiation markers, tight junction protein ZO1, cytokeratin 20 and the maturation of the apical plasma membrane. The arrangement of ZO1 into continuous lines in individual cells of the intermediate layer was already found after 10 min of chitosan application, when desquamation had just started. The appearance of the apical membrane changed from microvillar to typically scalloped within 20 min of regeneration, while complete arrangement of the cytokeratin 20 network took 60 min. These findings provide a new perspective on the rate of the differentiation process in the urothelium and make chitosan a new and a very controllable tool for studies on urothelial regeneration.

Toxicol Appl Pharmacol 2008; 232: 218-225.

Surface active stabilizer Tyloxapol in colloidal dispersions exerts cytostatic effects and apoptotic dismissal of cells

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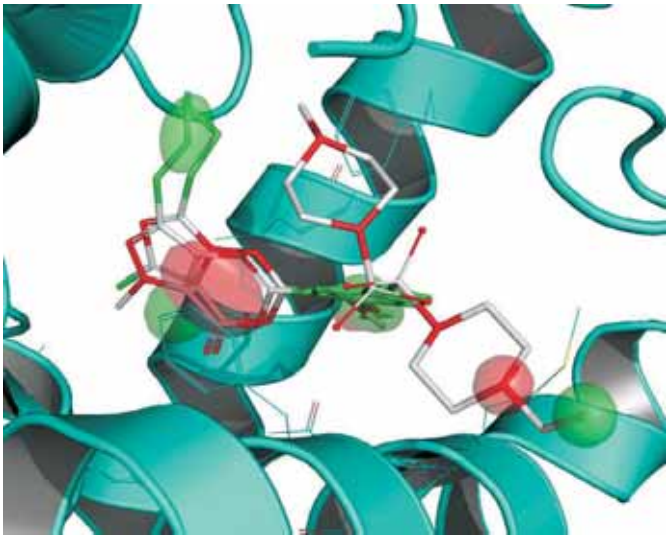
Solid lipid nanoparticles (SLN) have been praised for their advantageous drug delivery properties such as biocompatibility, controlled release and passive drug targeting. However, the cytotoxicity of SLN and their ingredients, especially over a longer time period, has not been investigated in detail. We examined the critical issues regarding the use of a surface active stabilizer Tyloxapol (Tyl) for the preparation of solid lipid particles (SLP) and their effects on cellular functions and viability. SLP composed of behenate, phospholipids and a stabilizer, Tyloxapol or Lutrol (Lut), were prepared by the lipid melt method, labeled with a fluorescent dye and tested on Jurkat or HEK293 cells. The nano-sized particles were rapidly internalized and exhibited cytoplasmic localization. Incubation of cells with SLP-Tyl resulted in a dose- and time-dependent cytostatic effect, and also caused moderate and delayed cytotoxicity. Tyloxapol solution or SLP-Tyl dispersion caused the detachment of HEK293 cells, a decrease in cell proliferation and alterations in cellular morphology. Cell cycle analysis revealed that, while the unfavourable effects of SLP-Tyl and Tyloxapol solution are similar initially, longer incubation results in partial recovery of cells incubated with the dispersion of SLP-Tyl, whereas the presence of Tyloxapol solution induces apoptotic cell death. These findings indicate that Tyloxapol is an unfavourable stabilizer of SLP used for intracellular delivery and reinforce the role of stabilizers in a design of SLP with minimal cytotoxic properties.

DHPLC (Denaturing high performance liquid cromatography Transgenomic (WAVE MD System)



Active site of phosphodiesterase 5 with superimposed low molecular weight inhibitors

Mol Genet Genomics 2007; 277: 391-401.

Heterogeneity in expression of the Escherichia coli colicin K activity gene cka is controlled by the SOS system and stochastic factors

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Phenotypic diversity provides populations of prokaryotic and eukaryotic organisms with the flexibility required to adapt to and/or survive environmental perturbations. Consequently, there is much interest in unraveling the molecular mechanisms of heterogeneity. A classical example of heterogeneity in *Escherichia coli* is the subset (3%) of the population that expresses the colicin K activity gene (*cka*) upon nutrient starvation. Here, we report on the mechanism underlying this variable response. As colicin synthesis is regulated by the LexA protein, the central regulator of the SOS response, we focused on the role of LexA and the SOS system in the variable *cka* expression. Real-time RT-PCR showed that the SOS system, without exogenous DNA damage, induces moderate levels of *cka* expression. The use of *cka–gfp* fusions demonstrated that modification of the conserved LexA boxes in the *cka* promoter region affected LexA binding affinity and the percentage of *cka–gfp* expressing cells in the population. A *lexA–gfp* fusion showed that the *lexA* gene is highly expressed in a subset of bacteria. Furthermore, c*ka–gfp* fusions cloned into higher copy plasmid vectors increased the percentage of *cka–gfp* positive bacteria. Together, these results indicate that the bistability in *cka* expression in the bacterial population is determined by (1) basal SOS activity, (2) stochastic factors and possibly (3) the interplay of LexA dimers at *cka* operator. Other LexA regulated processes could exhibit similar regulation.

Microb Cell Fact 2008; 7: str. 1-12.

Improved determination of plasmid copy number using quantitative real-time PCRfor monitoring fermentation processes

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BACKGROUND: Recombinant protein production in Escherichia coli cells is a complex process, where among other parameters, plasmid copy number, structural and segregational stability of plasmid have an important impact on the success of productivity. It was recognised that a method for accurate and rapid quantification of plasmid copy number is necessary for optimization and better understanding of this process. Lately, qPCR is becoming the method of choice for this purpose. In the presented work, an improved qPCR method adopted for PCN determination in various fermentation processes was developed. RESULTS: To avoid experimental errors arising from irreproducible DNA isolation, whole cells, treated by heating at 95 degrees C for 10 minutes prior to storage at -20 degrees C, were used as a template source. Relative quantification, taking into account different amplification efficiencies of amplicons for chromosome and plasmid, was used in the PCN calculation. The best reproducibility was achieved when the efficiency estimated for specific amplicon, obtained within one run, was averaged. It was demonstrated that the quantification range of 2 log units (100 to 10000 bacteria per well) enable quantification in each time point during fermentation. The method was applied to study PCN variation in fermentation at 25 degrees C and the correlation between PCN and protein accumulation was established. CONCLUSION: Using whole cells as a template source and relative quantification considering different PCR amplification efficiencies are significant improvements of the qPCR method for PCN determination. Due to the approaches used, the method is suitable for PCN determination in fermentation processes using various media and conditions.

Acta Chim Slov 2007; 54: 360-365.

Influence of the Media Composition on Behavior of pET Expression Systems

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pET expression systems (Novagen) are the strongest tool available for production of recombinant proteins in bacteria E. coli and have been widely used for many years. It is relatively difficult to control them due to their efficiency enabling formation of large amounts of recombinant proteins. Our work was focused on the influence of the media composition on the behavior of the pET3a expression system and we tried to select appropriate medium for inoculum preparation as well as appropriate production medium. We found out that media without glucose trigger unexpectedly high activity of lacUV5 promoter. Accumulation level of 15% of human granulocyte colony-stimulating factor (hG-CSF) in total cellular proteins was obtained in LBP medium (modified Luria-Bertani) without addition of IPTG inducer. Glucose addition into medium for inoculum preparation successfully represses expression of recombinant protein during inoculum preparation phase. Optimal optical density for high quality of inoculum is around OD600nm = 4,0, when culture is in the middle of exponential growth phase. Presence of glucose is required also in production medium. GYSP medium containing glucose, enables by 25% higher recombinant protein accumulation level than LBP medium without glucose. In contrast to LBP medium it enables comparably high recombinant protein accumulation level also without addition of antibiotic into the production medium.

Biotech Appl Biochem 2008; 49: 239-246.

New properties of inclusion bodies with implications for biotechnology

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Human G-CSF (granulocyte colony-stimulating factor) is a well-known biopharmaceutical drug being mostly produced by overexpression in *Escherichia coli*, where it appears in the form of IBs (inclusion bodies). Following our initial findings that properties of inclusion bodies strongly depend on the growth conditions used, especially growth temperature, we compared the characteristics of the G-CSF inclusion bodies prepared at two different temperatures, namely 42 and 25 °C. IBs formed at higher growth temperatures have properties similar to the usually described IBs, containing mainly denatured recombinant protein and being almost completely insoluble in aqueous solutions containing mild detergents or low concentrations of denaturants. In contrast, when produced at lower growth temperature of 25 °C, IBs show significantly different properties. Such IBs contain a significant proportion of G-CSF that is easily and directly extractable in the biologically active form, using non-denaturing solutions, which can be exploited for environmentally friendly biotechnological production. Irrespective of the production temperature, a significant decrease in IB volume was observed when transferring IBs from neutral to acidic (around 4) pH. Irreversible contraction of IBs at low pH was documented at the macro- and microscopic level using electron microscopy as a characterization tool. Together with volume decrease, a higher density, and thus decreased solubility, of IBs was observed at low pH, resulting in slower and less efficient extractability of the target protein.

Lett Appl Microbiol 2008; 46: 227-231.

Optimization of fermentation conditions for the expression of sweet-tasting protein brazzein in Lactococcus lactis

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AIMS: To improve the production of sweet-tasting protein brazzein in Lactococcus lactis using controlled fermentation conditions. METHODS AND RESULTS: The nisin-controlled expression system was used for brazzein expression. The concentration of nisin for induction and the optical density (OD) at induction were therefore optimized, together with growth conditions (medium composition, pH, aerobic growth in the presence of hemin). Brazzein was assayed with ELISA on Ni-NTA plates and Western blot. Use of the M-17 medium, containing 2.5% glucose, anaerobic growth at pH 5.9 and induction with 40 ng ml(-1) nisin at OD 3.0 led to an approx. 17-fold increase in brazzein per cell production compared to non-optimized starting conditions. Aerobic growth in the presence of hemin did not increase the production. CONCLUSIONS: Considerable increase in brazzein per cell production was obtained at optimized fermentation conditions. SIGNIFICANCE AND IMPACT OF THE STUDY: Optimized growth conditions could be used in application of brazzein expression in L. lactis. The importance of pH and OD at induction contributes to the body of knowledge of optimal recombinant protein expression in L. lactis. The new assay for brazzein quantification was introduced.

BioTechniques 2008; 44: 893-900.

Ultrasound in phage display: a new approach to nonspecific elution

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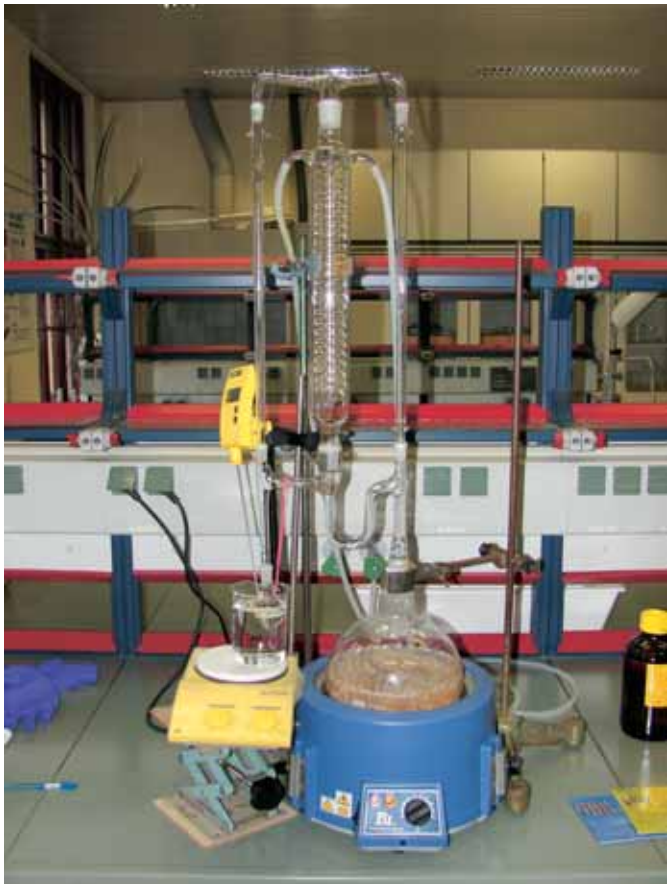
Libraries of phage-displayed random peptides are routinely used to identify target-binding peptides. Phages are commonly eluted in a nonspecific manner, especially if there are no available ligands of the particular target to use as competitors. However, the present study clearly demonstrates that nonspecific elution is not always able to break peptide-target interactions. To circumvent this we have developed an improved nonspecific elution strategy that uses ultrasound to release target-bound phages and enables selection of high-affinity clones in a single step.



Distillation with Clevenger apparatus

Work in biotechnological laboratory

Fungal cultures on plates

Eur J Cancer 2008; 44: 408–418.

Accessibility to targeted oncology drugs in Slovenia and selected European countries

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Aim: The aim of the study is to compare the accessibility to targeted oncology drugs in Slovenia and selected European countries.
Methods: Accessibility of targeted oncology drugs was assessed by using their sales data, expressed in mg per individual dying of the cancer for which the drug was indicated.
Results: The time of introduction of targeted oncology drugs in Slovenia was in most cases similar to the comparison countries, except for alemtuzumab and rituximab. The utilisation of targeted oncology drugs in Slovenia was in most cases lower than in other comparison countries. Ibritumomab had not been used in Slovenia until 2005, similar to France, Switzerland and UK. After 2003 the utilisation of trastuzumab in Slovenia started to rise substantially, approaching the average uptake in comparison countries.
Conclusion: The utilisation of targeted oncology drugs in Slovenia was in most cases lower than in comparison countries.

Zdrav Vestn 2007; 76: 529–38.

Admission of new oncological drugs to the Slovenian health care system and their accessibility to the patients

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Background The aim of the present study is to analyze the admission of anticancer drugs to the Slovenian healthcare system, and to evaluate patients' accessibility to these medications.
Methods Admission and accessibility to anticancer drugs was evaluated by analysing: differences in registration time among USA, selected member states of EU, and Slovenia; time from the registration to the first use in Slovenia; differences between the first use in Slovenia and the first use in selected member states of EU and by analysing the market of oncology drugs. The study included drugs of the ATC groups such as Antitumor medications (cytostatics) (ATC = L01) and Endocrine treatment (ATC = L02) that were used in Slovenia for the first time between 1999 to 2005. Registration data for Slovenia and EU was obtained from the registration documention of selected drugs at the Agency for Medicinal Products and Medical Devices of the Republic of Slovenia, and the European Agency for the Evaluation of Medicinal Products (EMEA). Registration data for USA was obtained from the registration documention of selected drugs at the Food and Drug Administration (FDA). The information upon the first use of drugs in Slovenia was acquired from the PharMIS system, and the information upon the first use of drugs in selected member states of EU was obtained from the study on patients' access to oncology drugs by Nils Wilking and Bengt Jönsson, performed from 2005. Data for the market analysis of oncology drugs was obtained from the PharmMIS system.
Results In previous years a delay in registration time between Slovenia and compared states was present for some oncology drugs. Along with the acceptance of Slovenia as a new member state of EU, on 1st May 2004, registration process in Slovenia became a part of the registration system of the European Agency for the Evaluation of Medicinal Products (EMEA). Majority of drugs had a time difference between the registration and the first use shorter than 10 months. Exceptions are drugs which were first used, mostly in a limited amount, before the acquisition of trade permit in Slovenia or through centralized procedure at EMEA such as oxaliplatin, irinotecan, capecitabine, imatinib mesylate. Average difference between the first use in Slovenia and selected member states of EU for the chosen oncology drugs decreased with years. In recent period oncology drugs were more quickly accessed by patients than in the past. The drug market continuously increased from 1999 to 2005, with an average increase of 30 M EUR per year. The largest increase was demonstrated for the antitumor medications and imunomodulators (ATC = L). In 1999 their market amounted to 10 M EUR or 3.9 % of the whole drug market, in 2005 it amounted to 38 M EUR or 8.6 % of the whole drug market. The market of the targeted drugs has been progressively increasing from year 2000 onwards, and amounted to 6 M EUR in 2005, which represents 39 % of the market share of Antitumor medications (cytostatics) (ATC = L01) group or 1.4 % of the market share for all of

the drugs. The reason for this progressive increase is attributed to the increased use of mainly three drugs: imatinib mesylate trastuzumab, and rituximab.
Conclusions The discrepancies in admission and access to newer oncology drugs between Slovenia and other member states of EU are becoming smaller. The market of oncology drugs is increasing significantly faster compared to the whole drug market, mainly due to the new targeted drugs

Int J Biol Markers 2008; 23: 161-168.

Cathepsin B, cathepsin H, cathepsin X and cystatin C in sera of patients with early-stage and inflammatory breast cancer

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Numerous studies have linked cathepsins and their inhibitor cystatin C to tumor invasion and metastasis. We examined whether cathepsin B, cathepsin H, cathepsin X and cystatin C could be detected in sera from women with early-stage or inflammatory breast cancer and whether they correlated with clinicopathological characteristics. Preoperative serum was obtained from 176 patients with early-stage breast cancer (tumor size <= 5 cm, negative lymph nodes) and 31 patients with inflammatory breast cancer. Cathepsin and cystatin C levels were measured by ELISA. The patient and tumor characteristics under study were age at diagnosis, menopausal status, tumor size, tumor grade, and steroid hormone receptor status. Serum cathepsin B levels were significantly lower in patients with poorly differentiated tumors. High cystatin C levels were associated with tumor size, postmenopausal status and patient age. Interestingly, significantly lower levels of cathepsin X and H were found in patients with inflammatory breast cancer, a trend also observed for cathepsin B and cystatin C. In conclusion, our results show a limited association of cathepsins B, H, X and cystatin C with established prognostic parameters. These data are promising and encourage future analysis of the clinical outcome of our patients in order to examine the potential prognostic value of these biomarkers. Further, this study indicates a role for cathepsin X and H in inflammatory breast cancer.

Pharmacogenomics 2008; 9: 539-549.

Cost-effectiveness of UGT1A1 genotyping in irinotecan colorectal cancer therapy

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Aim: The aim of the present study was to evaluate cost-effectiveness of UGT1A1 genotyping in second-line irinotecan therapy of metastatic colorectal cancer patients.
Methods: A decision analysis was used to compare standard therapy to alternative strategies based on UGT1A1 genotyping from the USA healthcare payer perspective. The effectiveness outcome was severe neutropenia occurrence, and number of life-years gained.
Results & Conclusions: Genotyping in combination with a subsequent reduction of initial irinotecan dose for UGT1A1 7/7 genotype patients could be cost-saving for the population of African and Caucasian origin. On the contrary, UGT1A1 genotyping was not cost-effective for the population of Asian ancestry. Nevertheless, the prophylactic use of granulocyte-colony stimulating factors in UGT1A1 7/7 genotype patients was not cost-effective in any population group.

Cancer Lett 2007; 248: 192-197.

Cystatin C as a potential marker for relapse in patients with non-Hodgkin B-cell lymphoma

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The concentration of cysteine protease inhibitor cystatin C was determined in sera from 59 patients with non-Hodgkin B-cell lymphoma using ELISA. The sera from 43 age and sex matched healthy blood donors served as controls. Cystatin C was significantly increased in sera of patients without therapy (mean 1136 +/- SE 105.7 ng/ml, p = 0.00001) and with therapy (mean 1073 +/- 52 ng/ml, p = 0.001) compared to controls (mean 819 +/- 28 ng/ml). The highest levels were determined in sera of patients with a relapse (mean 1680 +/- 196 ng/ml). By using immunofluorescence staining and confocal microscopy we determined immature dendritic cells as a major population of cystatin C positive cells in affected lymph nodes. Our study reports for the first time that cystatin C is a potential marker for relapse in patients with non-Hodgkin B-cell lymphoma.

Chem-Biol Interact 2009, 178, 158-164.

Derivatives of pyrimidine, phthalimide and anthranilic acid as inhibitors of human hydroxysteroid dehydrogenase AKR1C1

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Human hydroxysteroid dehydrogenase (HSD) AKR1C1, a member of the aldo-keto reductase superfamily, functions mainly as a 20α-HSD. It catalyzes reduction of a potent progesterone to a weak 20á-hydroxyprogesterone and the reduction of 3α,5α-tetrahydro-progesterone (5alpha-THP, allopregnanolone) to 5α-pregnane-3α, 20α-diol. AKR1C1 thus diminishes the levels of progesterone and 5α-THP in peripheral tissue. Progesterone inhibits proliferation, stimulates differentiation of endometrial cells and is also important for maintaining pregnancy, while 5α-THP allosterically modulates the activity of the gama-aminobutyric acid (GABA) receptor. Inhibitors of AKR1C1 are thus very interesting as potential agents for the treatment of endometrial cancer, endometriosis, as well as other diseases such as premenstrual syndrome, catamenial epilepsy, and depressive disorders.
We synthesized a series of pyrimidine, phthalimido and athranilic acid derivatives, and examined their AKR1C1 inhibitory properties. A common AKR substrate 1-acenaphthenol was used to monitor NAD^+-dependent oxidation catalyzed by AKR1C1. The most potent inhibitors were pyrimidine derivative *N*-benzyl-2-(2-(4-methoxybenzyl)-6-oxo-1,6-dihydropyrimidin-4-yl)acetamide (K_i = 17 μM) and anthranillic acid derivative 2-(((2',3-dichlorobiphenyl-4-yl)carbonyl)(methyl)amino)benzoic acid (K_i = 33 μM) both acting as non-competitive inhibitors.

Mol Cell Endocrinol 2009, 301, 245-250.

Discovery of new inhibitors of aldo-keto reductase 1C1 by structure-based virtual screening

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Aldo-keto reductase 1C1 is a hydroxysteroid dehydrogenase that inactivates progesterone by converting it to 20α-hydroxyprogesterone. It also inactivates 3α,5α-tetrahydroprogesterone, an allosteric modulator of the -aminobutyric acid receptor that has anaesthetic, analgesic, anxiolytic and anticonvulsant effects. Inhibitors of aldo-keto reductase 1C1 are thus very interesting as potential agents for the treatment of endometrial cancer, premenstrual syndrome, catamenial epilepsy, and depressive disorders, and for the maintenance of pregnancy. We have used the molecular docking program eHiTS for virtual screening of 1,990 compounds from the National Cancer Institute "Diversity Set". Fifty compounds with the highest predicted binding energies were then evaluated *in vitro*. Three structurally diverse hits were obtained that inhibit aldo-keto reductase 1C1 in the low micromolar range of IC_{50} values. These hits represent promising starting points for structural optimization in hit-to-lead development.

Leukemia, in press

Heterozygosity at the TPMT gene locus, augmented by mutated MTHFR gene, predisposes to 6-MP related toxicities in childhood ALL patients

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The clinical relevance of 6-mercaptopurine dose reduction in prevention of thiopurine-induced toxicity is well established in patients homozygous for low activity alleles of thiopurine S-methyltransferase (TPMT) but uncertain in heterozygous individuals. 6-mercaptopurine (6-MP) is an antimetabolite widely used in the treatment of acute lymphoblastic leukemia (ALL). Its two main modes of action are cytotoxicity, achieved through incorporation of thioguanine nucleotides (TGN) into DNA and RNA, and inhibition of *de novo* purine synthesis by methyl-thioinosine monophosphate (meTIMP). Metabolic pathways activating 6-MP compete with inactivation pathways catalyzed by xanthine oxidase (XO) and TPMT, whose activity is a good prediction factor for the toxicity and effectiveness of the antileukemic thiopurine therapy.

Biochimie 2007; 89: 63-71.

His164 regulates accessibility to the active site in fungal 17β-hydroxysteroid dehydrogenase

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17β-Hydroxysteroid dehydrogenase from the fungus *Cochliobolus lunatus* (17β-HSDcl) is an NADPH-dependent member of the short-chain dehydrogenase/ reductase superfamily. To study the catalytic properties of this enzyme, we prepared several specific mutations of 17β-HSDcl (Tyr167Phe, His164Trp/Gly, Tyr212Ala). Wild-type 17β-HSDcl and the 17β-HSDcl mutants were evaluated by chromatographic, kinetic and thermodynamic means. The Tyr167Phe mutation resulted in a complete loss of enzyme activity, while substitution of His164 with Trp and Gly both resulted in higher specificity number (*V/K*) for the steroid substrates, which are mainly a consequence of easier accessibility of steroid substrates to the active-site hollow under optimized conditions. The Tyr212Ala mutant showed increased activity in the oxidative direction, which appears to be a consequence of increased NADPH dissociation. The kinetic characterizations and thermodynamic analyses also suggest that His164 and Tyr212 in 17β-HSDcl have a role in the opening and closing of the active site of this enzyme and in the discrimination between oxidized and reduced coenzyme.

Leuk Res 2009, in press

5,10-Methylenetetrahydrofolate reductase (MTHFR) low activity genotypes reduce the risk of relapse-related acute lymphoblastic leukemia (ALL)

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The reported correlation of defects in 5,10-methylenetetrahydrofolate reductase (MTHFR), the key enzyme of folatemetabolism, with modulated risk foracute lymphoblastic leukemia (ALL) is ambiguous. We have elucidated the influence of MTHFR genotype on ALL development and relapse in 140 Slovenian pediatric ALL patients and 183 healthy controls. A decreased proportion of lowactivity MTHFR genotypes were found in a group of ALL patients with relapses compared to healthy controls ($p = 0.022$) and ALL cases without relapse ($p = 0.027$). Mutations in the MTHFR gene decrease the onset risk of ALL with relapse in the setting of no folate supplementation in pregnancy, butnot of relapse-free ALL.

Cancer Lett 2008; 267: 75-84.

Monoclonal antibody to cytokeratin VKIALEVEIATY sequence motif reduces plasminogen activation in breast tumour cells

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Cytokeratins (CKs) are the main structural proteins of epithelial cells. Although they mainly form cytoplasmic structures, they are also localized at the plasma membrane or secreted from the cells. Some CKs are over-expressed in tumor cells and are used as diagnostic and prognostic biomarkers. A stable hybridoma cell line producing anti-cytokeratin monoclonal antibody (anti-CK MAb) was prepared after immunizing mice with breast cancer MCF-7 cell lysate. As shown by 2D electrophoresis, immunoblotting and mass spectroscopy, the monoclonal antibody recognizes ail epitope oil CK1, CK2, CK8, CK10 and CK18 in MCF-7 cells. To identify the binding site of the antibody three peptides of 12 amino acids were synthesized, each overlapping a 27 amino acid consensus sequence of the recognized CKs. Anti-CK MAb expressed high affinity for it dodecapeptide with the sequence VKIALEVEIATY, localized in the CK alpha-helical B2 domain, as shown by ELISA and surface plasmon resonance. Treatment of MCF-7 cells by anti-CK MAb impaired plasminogen activation and consequently invasiveness of the cells. Our results show that, besides their use in diagnosis, anti-cytokeratin antibodies Could be used in therapy of invasive breast cancer.

Mol Cell Endocrinol 2009; 301: 47-50.

Mutations that affect coenzyme binding and dimer formation of fungal 17beta-hydroxysteroid dehydrogenase

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The 17β-hydroxysteroid dehydrogenase from the fungus *Cochliobolus lunatus* (17β-HSDcl) is an NADPH-dependent member of the short-chain dehydrogenase/reductase superfamily, and it functions as a dimer that is composed of two identical subunits. By constructing the appropriate mutants, we have examined the M204 residue that is situated in the coenzyme binding pocket, for its role in the binding of the coenzyme NADP(H). We have also studied the importance of hydrophobic interactions through F124, F132, F133 and F177 for 17β-HSDcl dimer formation. The M204G substitution decreased the catalytic efficiency of 17β-HSDcl, suggesting that M204 sterically coerces the nicotinamide moiety of the coenzyme into the appropriate position for further hydride transfer. Phenylalanine substitutions introduced at the dimer interface produced inactive aggregates and oligomers with high molecular masses, suggesting that these hydrophobic interactions have important roles in the formation of the active dimer.

Ann Hematol 2008; 87: 131-137.

Platelet glycoprotein IIIa gene expression in normal and malignant megakaryopoiesis

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The platelet glycoprotein GPIIb/IIIa functions as a receptor for fibrinogen in platelet aggregation process and is an example of an early megakaryocytic marker. One of a chronic myeloproliferative disorder, essential thrombocythemia, is caused by abnormal megakaryopoiesis. Due to the lack of reliable method for the diagnosis of that disease and the importance of GPIIIa as a marker for identifying early megakaryocytes, the expression level of GPIIIa in mononuclear and CD34+ cells and during megakaryopoiesis was compared between normal individuals and patients with essential thrombocythemia. For this purpose, surface markers GPIIIa and CD34 were analyzed with flow cytometer, and GPIIIa expression level was measured with real-time polymerase chain reaction (PCR) method. Mononuclear and CD34+ cells from normal individuals and patients were isolated, analyzed, and seeded into serum-free medium Stemspan™ Medium enriched with IL-6, IL-3, thrombopoietin, and stem cell factor. The difference between normal individuals and patients was noticed in the expression level of GPIIIa in the CD34+ cells and in the time course of cell surface markers. CD34+ cells from patients has 33% higher of GPIIIa antigens on the surface and 34% higher GPIIIa messenger RNA (mRNA) expression level. The negative effect of IL-3 on the maturation of megakaryocytes was not noticed; there were 56.46% of megakaryoblasts at the end of the cultivation, and after 14 days of culturing, 111.09 times increase of GPIIIa mRNA in patients was detected. This study is therefore offering the method that could serve as reliable tool for discriminating ET from other similar myeloproliferative disorders.

Value in Health 2008; 11: 139-148.

Probabilistic Cost-Effectiveness Modeling of Different Breast Cancer Screening Policies in Slovenia

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Objectives: To determine the most cost-effective screening policy for population-based mammography breast cancer screening in Slovenia using probabilistic sensitivity analysis.
Methods: A time-dependent Markov model for breast cancer was constructed. General principles of cost-effectiveness analysis with multiple strategies were used to compare the costs and effects of 36 different screening policies. Using probability distributions for model parameters, the true effect of uncertainty across model input parameters on expected costs and effects was explored. The results from probabilistic simulation analysis are presented in a form of cost-effectiveness acceptability curves with cost-effectiveness acceptability frontier.
Results: With the presented analysis, it was shown that a 1-year screening interval in population breast cancer screening would produce less benefits at higher costs than less intensive screening and that a 2-year interval would be cost-effective only at high values of society's willingness to pay per quality-adjusted life-year (QALY). Therefore, the optimal screening policy should be chosen among 3-year-interval policies.
Conclusions: Based on commonly quoted thresholds of society's willingness to pay per QALY of $50,000, the optimal approach in the Slovenian population would be screening women aged from 40 to 80 years every 3 years.

Biochim Biophys Acta 2009; 1790: 173-181.

Purification, characterization and cloning of a ricin B-like lectin from mushroom Clitocybe nebularis with antiproliferative activity against human leukemic T cells

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BACKGROUND: Lectins are a diverse group of carbohydrate-binding proteins exhibiting numerous biological activities and functions. METHODS: Two-step serial carbohydrate affinity chromatography was used to isolate a lectin from the edible mushroom clouded agaric (Clitocybe nebularis). It was characterized biochemically, its gene and cDNA cloned and the deduced amino acid sequence analyzed. Its activity was tested by hemagglutination assay and carbohydrate-binding specificity determined by glycan microarray analysis. Its effect on proliferation of several human cell lines was determined by MTS assay. RESULTS: A homodimeric lectin with 15.9-kDa subunits agglutinates human group A, followed by B, O, and bovine erythrocytes. Hemagglutination was inhibited by glycoprotein asialofetuin and lactose. Glycan microarray analysis revealed that the lectin recognizes human blood group A determinant GalNAcα1–3(Fucα1–2)Galβ-containing carbohydrates, and GalNAcβ1–4GlcNAc (N,N'-diacetyllactosediamine). The lectin exerts antiproliferative activity specific to human leukemic T cells. CONCLUSIONS: The protein belongs to the ricin B-like lectin superfamily, and has been designated as C. nebularis lectin (CNL). Its antiproliferative effect appears to be elicited by binding to carbohydrate receptors on human leukemic T cells. GENERAL SIGNIFICANCE: CNL is one of the few mushroom ricin B-like lectins that have been identified and the only one so far shown to possess immunomodulatory properties.

Connect Tissue Res 2008; 49: 693-696.

Role of cysteine cathepsins in matrix degradation and cell signalling

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Cysteine cathepsins participate in extracellular matrix (ECM) degradation and remodelling and thus influence important cellular processes such as cell transformation and differentiation, motility, adhesion, invasion, angiogenesis, and metastasis. Also, cathepsins are involved in cell signalling and are capable of activating specific cell receptors and growth factors or liberating them from the ECM. In this review we emphasize recent studies on cathepsins in regard to ECM degradation and cell signalling.

Int J Radiat Oncol Biol Phys 2007; 68: 1335-1341.

Stefin a and stefin B : markers for prognosis in operable squamous cell carcinoma of the head and neck

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The aim of this study was to test the hypothesis about the protective role of high stefin A and stefin B concentrations in operable carcinoma of the head and neck. METHODS AND MATERIALS: Stefins A and B concentrations were measured in tissue cytosols of nontumorous mucosa and primary tumors from 92 patients. For quantitative analysis of stefins in tumor cytosols, commercially available enzyme-linked immunosorbent assays were used. RESULTS: Stefin A was upregulated in 53 patients (higher concentrations were measured in tumor samples than in nontumorous mucosa) and was downregulated in 39 patients. The corresponding numbers for stefin B were 49 and 43, respectively. A significantly higher proportion of downregulated cases were found among patients with disease re-appearance. In the Cox model, high stefin A concentrations appeared as independent predictors for favorable disease-free survival. Assuming a "broken stick" model, a significant increase in the recurrence rate after the threshold of 1063 ng/mgp (the 64th percentile in the group) was found, the hazard ratio reaching 3% of the reference value with doubling of the level of stefin A. These results were reconfirmed after pooling the data with two historical data sets into a uniform series involving 182 patients. CONCLUSIONS: A group of patients at high risk for disease progression was identified, characterized by the downregulated stefin A protein in the tumor compared with the nontumorous mucosa. Stefin A was recognized as a promising candidate marker for prognosis in patients with operable carcinoma of the head and neck.

Nucl Med Comm, 2008; 29: 1059-1065.

^{99m}Tc-labelled rituximab, a new non-Hodgkin's lymphoma, imaging agent: first clinical experience

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Objective This study was performed to explore the possibility of using 99mTc-rituximab as an imaging agent to assess expression of CD20 antigen in patients with B-cell non-Hodgkin's lymphoma (NHL) before (radio) immunotherapy, for staging and subsequent evaluation of remission of NHL. Methods Rituximab was purified from Mabthera and photoactivated by ultravioletlight. The irradiated solution was aliquoted and labelled with pertechnetate. The effectiveness of the labelling method was evaluated by determination of the number of free thiol groups per photoreduced antibody, radiochemical purity determination and in-vitro stability. Immunoreactivity of99mTc-rituximab was assessed on Ramos cells using a direct binding assay. Ten patients (age 31-70 years, mean 50 years) were included, nine with CD20+ B-cell NHL and one with CD20- NHL. Whole-body and single photon emission computed tomography images were taken 1, 3, 6 and 20 h postinjection of 99mTc-rituximab. Scintigraphic results were compared with computerized tomography (CT) findings. Results In all cases radiochemical purity over 95% was observed with preserved affinity for CD20 antigen. In all patients expected activity was seen in the blood pool, liver, kidneys and spleen. Pathological, moderately to markedly.

Cancer Genet Cytogenet 2008; 187: 28-33.

The presence of the CYP11A1 (TTTTA)6 allele increases the risk of biochemical relapse in organ confined and low grade prostate cancer

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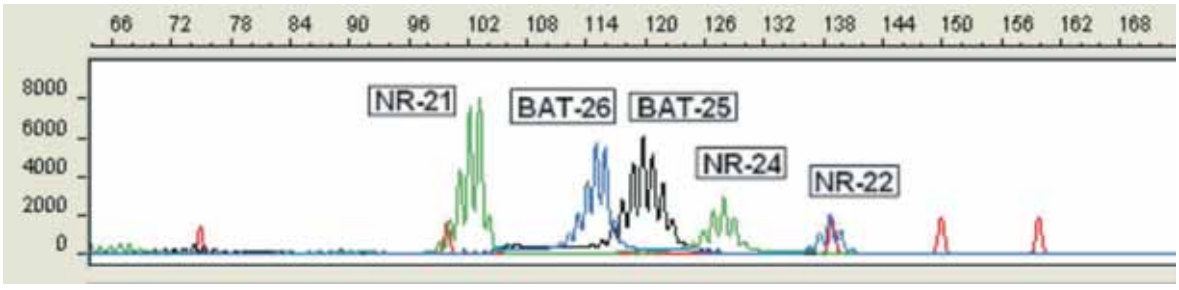
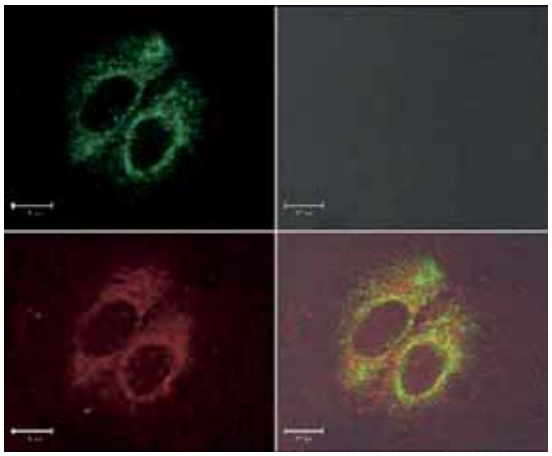
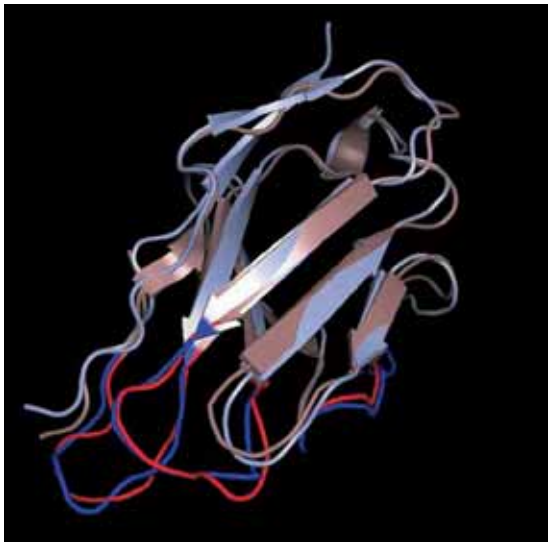
The involvement of the CYP11A1 gene in the synthesis of androgens makes it a compelling candidate for various hormone-dependent diseases, including prostate cancer. A microsatellite polymorphism (TTTTA)n in the promoter regionof the CYP11A1 gene has been reported to be associated with an increasedrisk of metastatic and high-grade prostate cancer. In the present study of 110 prostate cancer patients and 96 population controls, we examined the association between the CYP11A1 (TTTTA)n polymorphism and prostate cancer risk, aggressiveness, and incidence of biochemical relapse after prostatectomy. We have also evaluated the potential of the (TTTTA)n polymorphism as a microsatellite marker for the detection of genomic instability in prostate cancer. A strong association of the genotype containing the (TTTTA)6 allele with the occurrence of biochemical relapse after prostatectomy in patients with organ confined prostate cancer ($p<0.0001$), as well as in patients with low-grade prostate cancer ($p=0.002$) orboth ($p<0.0003$) was determined. The incidence of biochemical relapse in patients with organ confined and low-grade prostate cancer in our study group was 22%, but increased to 50% in carriers of the (TTTTA)6 allele. Our findingsalso suggest (TTTTA)n instability as a potential marker for the detection of early events in carcinogenesis.



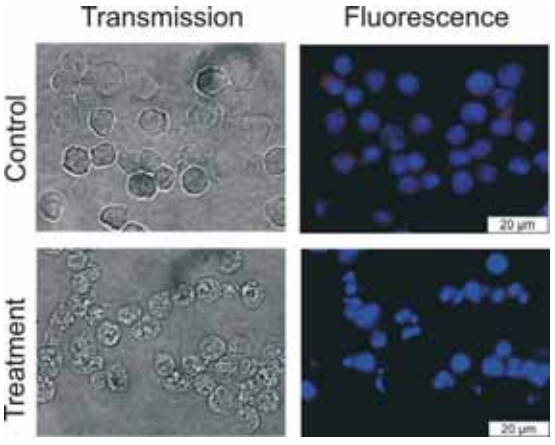
Microsatellite analysis of prostate cancer specimens by fluorescence multiplex polymerase chain reaction.



Co-localisation of cytokeratin antigens on the surface of nonpermeabilised MCF-10A neoT cells (fluorophores: Alexa 488 - green, Alexa 546 - red).



Superimposed model structures of the anti-cytokeratin antibody light chain. Both structures were designed in silico based on the primary amino acid sequence.



Photomicrophaphs showing untreated (control) WEHI231 cells and apoptosis after treatment with a serine protease inhibitor (treatment).

J Med Chem 2008; 51: 5617–5629.

3,4-Dihydro-2H-1,4-benzoxazine Derivatives Combining Thrombin Inhibitory and Glycoprotein IIb/IIIa Receptor Antagonistic Activity as a Novel Class of Antithrombotic Compounds with Dual Function

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Abstract: 3,4-Dihydro-2*H*-1,4-benzoxazine derivatives possessing both thrombin inhibitory and glycoprotein IIb/IIIa (GPIIb/IIIa) receptor antagonistic activities were obtained by combining mimetics of the *D*-Phe-Pro-Arg pharmacophore of thrombin inhibitors and the Arg-Gly-Asp pharmacophore of GPIIb/IIIa receptor antagonists in the same low molecular weight peptidomimetic compound. Systematic variation of the position of substituents around the 3,4-dihydro-2*H*-1,4-benzoxazine nucleus, the distance between the carboxylate and amidine moieties, together with additional substituents to fill the thrombin S_2 and S_3 pockets resulted in compounds displaying submicromolar inhibition constants (K_i) for thrombin and submicromolar IC_{50} for inhibition of binding of fibrinogen to platelet GPIIb/IIIa receptor. Some of these compounds, such as **17a**, **17b**, **17d**, and **17h** possessing a well balanced activity at both targets, are a good starting point for further optimization. Incorporation of anticoagulant and platelet antiaggregatory activity in the same molecule constitutes a promising approach toward novel antithrombotic agents.

J Med Chem 2008; 51: 2863-2867.

Novel potent and selective thrombin inhibitors based on a central 1,4-benzoxazin-3(4H)-one scaffold

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Novel thrombin inhibitors with the central 1,4-benzoxazine-3(4*H*)-one scaffold, benzamidine P_1 arginine side chain mimetic and various P_3 moieties are described. 3-(Benzyl(2-(4-carbamimidoylbenzyl)-4-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-7-yl)amino)-3-oxopropanoic acid (**7b**), the most potent compound in the series, exhibited a K_i of 2.6 nM in vitro for thrombin and high selectivity against trypsin and factor Xa.

Curr Pharm Des 2007; 13: 287-312.

Recent advances in serine protease inhibitors as anticoagulant agents

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The drawbacks and limitations of existing anticoagulant therapy which may result in serious adverse effects and a high mortality rate, have given rise to many anticoagulant development programmes in the last decade, focusing mainly at development of thrombin and FXa low-molecular weight inhibitors. A detailed understanding of blood coagulation pathways, functioning of the serine proteases thrombin, FXa, FVIIa and FIXa and elucidation of their crystal structures resulted in many potent compounds, among which some have entered the clinical phase or have been approved for use in clinical practice. Recently, the focus of anticoagulant research turned to inhibition of the TF:FVIIa complex, with some promising clinical candidates on the horizon. This article provides an overview of the current development status of serine protease inhibitors as anticoagulants, including new trends such as dual coagulation factor inhibitors.

Pharmazie, 2007; 62: 243-254.

Design, Synthesis and Molecular Modelling of 1-Amidinopiperidine Thrombin Inhibitors

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Design, synthesis and biochemical evaluation of a series of novel non-covalent thrombin inhibitors with a 1-amidinopiperidine moiety are presented. Replacement of the planar benzamidine group in azaphenylalanine derivatives with 1-amidinopiperidine resulted in lower activity but higher selectivity for this type of compounds. The binding conformation of inhibitors in the active site of thrombin was revealed by molecular modelling studies.

Bioorg Med Chem 2008; 16: 209-221.

Novel cholesterol biosynthesis inhibitors targeting human lanosterol 14α-demethylase (CYP51)

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Novel cholesterol biosynthesis inhibitors, a group of pyridylethanol(phenylethyl)amine derivatives, were synthesized. Sterol profiling assay in the human hepatoma HepG2 cells revealed that compounds target human lanosterol 14α-demethylase (CYP51). Structure–activity relationship study of the binding with the overexpressed human CYP51 indicates that the pyridine binds within the heme binding pocket in an analogy with the azoles.

J Pharm Biomed Anal 2009; 49: 295-303.

Stability of new potential ACE inhibitor in the aqueous solutions of different pH

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Angiotensin-converting enzyme (ACE) inhibitors are a group of active substances binding to an active site of ACE. Many authors who studied the structure activity relationship suggested the structural elements needed for a potent ACE inhibitor. While many authors studied the activity of ACE inhibitor substances only a few structure stability studies have been presented. In this paper the stability properties of molecule xPRIL were studied by determination of degradation path and rate of degradation in aqueous solutions with different pH (2.0, 6.8 and 12.0) and temperatures (40, 60 and 80 C). The degradation of molecule through two main degradation paths was identified and confirmed by liquid chromatography and mass spectroscopy (LC-MS). Stability properties of xPRIL were determined in a stability study evaluated by high-performance liquid chromatography (HPLC). The first order kinetics of degradation reaction of xPRIL and Arrhenius equations for each pH were determined at observed conditions. xPRIL showed the highest stability at pH 2 solution. The degradation kinetics of xPRIL was compared to the degradation kinetics of enalapril maleate (EM) and perindopril (PER) in bio relevant solutions with pH 2.0 and 6.8. In addition to the stability study of xPRIL the forced degradation study of all three molecules at rigorous conditions was conducted. From the obtained results the structural element having the highest influence on stability properties of the studied molecules was identified. The fragmentation paths of xPRIL, its cyclization degradation product and its hydrolysis degradation product were identified and confirmed by MS/MS method. (c) 2008 Elsevier B.V. All rights reserved.

Physiol Genom 2007; 31: 216-227.

TNF-αinterferes with lipid homeostasis and activates acute and proatherogenic processes

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The interaction between disrupted lipid homeostasis and immune response is implicated in the pathogenesis of several diseases, but the molecular bridges between the major players are still a matter of controversy. Our systemic study of the inflammatory cytokine tumor necrosis factor-alpha (TNF-α) in the livers of mice exposed to 20-h cytokine/fasting for the first time shows that TNF-αinterferes with adaptation to fasting and activates harmful proatherogenic pathways, partially through interaction with the insulin-Insig-sterol regulatory element binding protein (Srebp) signaling pathway. In addition to the increased expression of acute-phase inflammatory genes, the most prominent alterations represent modified lipid homeostasis observed on the gene expression and metabolite levels. These include reduction of HDL-cholesterol, increase of LDL-cholesterol, and elevated expression of cholesterogenic genes, accompanied by increase of potentially harmful precholesterol metabolites and suppression of cholesterol elimination through bile acids, likely by farnesoid X receptor-independent mechanisms. On the transcriptional level, a shift from fatty oxidation toward fatty acid synthesis is observed. The concept of the influence of TNF-α on the Srebp regulatory network, followed by downstream effects on sterol metabolism, is novel. Observed acute alterations in lipid metabolism are in agreement with chronic disturbances found in patients.

J Mol Model 2007; 13: 247-254.

Thrombin inhibitors with novel P1 binding pocket functionality. free energy of binding analysis

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3 Lek, d.d., Pharmaceutical and Chemical Company, 1526 Ljubljana, Slovenia

The high incidence of thrombembolic diseases justifies the development of new antithrombotics. The search for a direct inhibitor has resulted in the synthesis of a considerable number of low molecular weight molecules that inhibit human α-thrombin potently. However, efforts to develop an orally active drug remain in progress as the most active inhibitors with a highly basic P1 moiety exhibit an unsatisfactory bioavailability profile. In our previous work we solved several X-ray structures of human α-thrombin in complexes with (1) novel bicyclic arginine mimetics attached to the glycylproline amide and pyridinone acetamide scaffold and (2) inhibitors with a novel aza scaffold and with charged or neutral P1 moieties. In the present contribution, we correlate the structures of the complex between these inhibitors and the protein with the calculated free energy of binding. The energy of solvation was calculated using the Poisson–Boltzmann approach. In particular, the requirements for successful recognition of an inhibitor at the protein's active site pocket S1 are discussed.

Clin Chem Lab Med 2007; 45: 1542-1548.

Utility of PFA-100((R)) closure time vs. optical aggregometry in assessing the efficacy of platelet membrane glycoprotein IIb/IIIa antagonists in vitro

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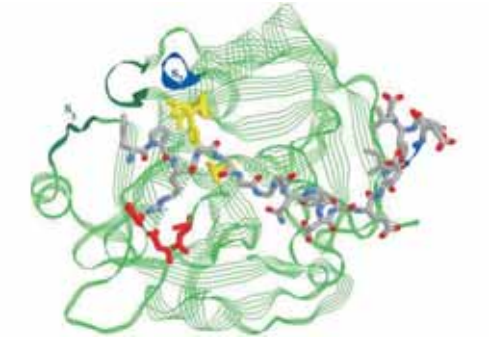
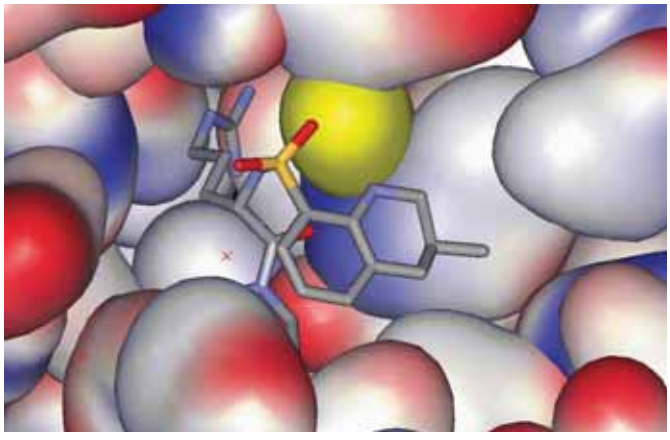
Closure time measured by a platelet function analyser (PFA-100®) was tested for its utility in assessing the efficacy of platelet membrane glycoprotein IIb/IIIa antagonists in vitro in comparison to optical platelet aggregometry. Three known glycoprotein IIb/IIIa antagonists (H-Arg-Gly-Asp-Ser-OH: RGDS, tirofiban and eptifibatide) and six new peptidomimetic glycoprotein IIb/IIIa antagonists (DKT-59, DPS-172, SMA-101, SMA-104, SMA-179, SKN-191), were tested. The concentration of antagonist which doubled closure time in collagen/ADP and collagen/epinephrine cartridges (IC200) or decreased ADP or collagen-induced platelet aggregation by 50 % (IC50) was taken to assess the efficacy of the glycoprotein IIb/IIIa antagonist in inhibiting platelet function. Closure time represents a fast, simple and sensitive method of assessing glycoprotein IIb/IIIa antagonism in vitro, is comparable to optical aggregometry, and suitable for testing larger numbers of glycoprotein IIb/IIIa antagonists.

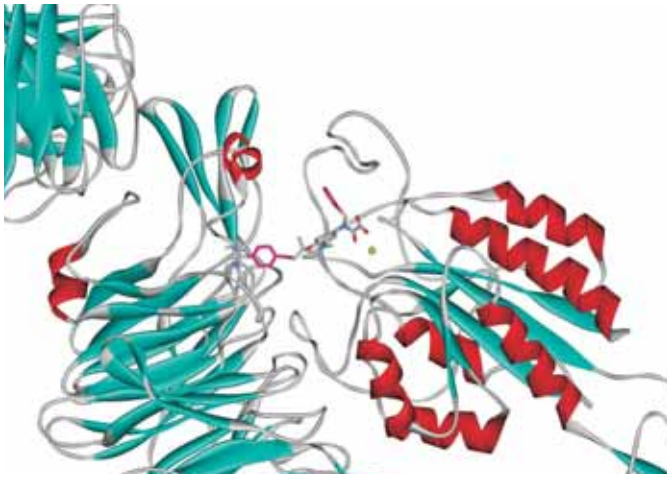


Thrombin in complex with hirudin



Low molecular weight azaphenylalanine inhibitor in the active site of thrombin.

Dual antithrombotic compound docked to the thrombin active site



Dual antithrombotic compound docked to the GPIIb/IIIa (fibrinogen receptor)

Eur J Pharm Biopharm 2008; 69: 698–707

Effect of calcium ions on the gelling and drug release characteristics of xanthan matrix tablets

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Xanthan is a well-known biopolymer. It is an anionic polysaccharide, whose primary structure depends on the bacterial strain and fermentation conditions. Xanthan was extensively studied in combination with galactomannans, and over 90 patents cover the technology of this preparation. Our aim was to investigate the relation between the physical properties of a xanthan matrix in the absence or presence of calcium ions and its influence on the release of pentoxifylline. The release of pentoxifylline from xanthan tablets in purified water was shown to be very slow and governed by the process of polymer relaxation. The presence of calcium ions significantly increased the drug release, changing the release mechanism into a more diffusion controlled one. Xanthan matrices showed substantially faster and more extensive swelling in water than in the presence of Ca^{2+} ions. Surprisingly, negative correlation between drug release and degree of swelling was obtained for xanthan: the higher the swelling, the slower the drug release. Higher ionic strength led to lower erosion of xanthan tablets, and the gel layers formed were more rigid and of firmer texture, as shown by rheological experiments and textural profiling. The results indicate that the presence of Ca^{2+} ions in the solution or in matrices does not cause crosslinking of xanthan polymers, but causes charge screening of ionized groups on the trisaccharide side chains of xanthan, leading to lower inter-molecular repulsion and changing water arrangement. The understanding of the parameters influencing drug release leads to the conclusion that xanthan is suitable for controlled release formulations, especially with the incorporation of certain small counterions.

Int J Pharm 2007; 330: 164-174.

Novel hybrid silica xerogels for stabilization and controlled release of drug

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Purpose The goal was to show that incorporation of a model drug into a porous solid matrix with small enough pores should lead to composites in which the drug would be in the amorphous rather than in the crystalline state. Due to spatial constraints, the amorphous state was expected to be temporally highly stable. Methods As a porous 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). Results In all composites the amorphous state of nifedipine was proven using several analytical methods. The amorphicity was preserved for at least several months. Drug incorporation into purely TEOS-based silica decreased significantly the release rate. Loosening the structure by addition of BTSE, while preserving the amorphicity, increased the drug dissolution rate. The dissolution behaviour was explained using a combination of the Noyes–Whitney and power law model. Conclusion The observed release patterns could be interesting for therapies requiring a high initial drug concentration in blood plasma, followed by a slower release rate of the remaining drug.

e-Polymers 2009, in press

The reflection of the texture of swollen polymer matrix on the release of incorporated substance

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Our aim was to investigate the texture of hydrated biopolymer matrices that are now being considered in the design of pharmaceutical controlled-release dosage forms, in order to determine their influence on the release of an active compound. Prolonged release of pentoxifylline, a highly soluble drug, is needed for once-daily administration to achieve its therapeutic effect. For this purpose, pentoxifylline was incorporated in a polymer matrix made of a combination of xanthan and locust bean gum (XLBG), both of which are of biotechnological origin. Different methods were used to investigate the interplay of the XLBG gel structure characteristics in the absence and presence of 200 mM $CaCl_2$ on pentoxifylline release: drug-release studies, determination of swelling, erosion, and visco-elasticity of the gel, as well as its texture analysis and microscopic imaging. From the results obtained, the following conclusions can be drawn: the pentoxifylline release from XLBG matrices in water was prolonged for 24 h whereas from the control lactose formulation was completed within 30 min. The presence of Ca^{2+} ions in water resulted in faster pentoxifylline release, in spite of less swelling and erosion. However, the rheology, texture analysis and scanning electron microscopy revealed that in the presence of the Ca^{2+} ions the gel layer of the XLBG was more cohesive and thinner, as the attraction for water molecules was lower due to the condensation of counter-ions on the xanthan carboxylic-moieties, and consequently greater interpolymer interactions. Therefore, relatively larger amounts of free water molecules were available within the XLBG hydrogel in the presence of Ca^{2+}, allowing faster drug dissolution and diffusion. Here, the presence of Ca^{2+} ions had a completely opposite effect on XLBG gel structure and drug release in comparison with other more investigated matrix polymers like alginate or non-ionic cellulose ethers. A firm matrix structure that is accompanied by low swelling and erosion cannot guarantee a more prolonged drug release.

Int J Pharm 2007; 343: 131-140.

Vitrification from solution in restricted space: Formation and stabilization of amorphous nifedipine in a nanoporous silica xerogel carrier

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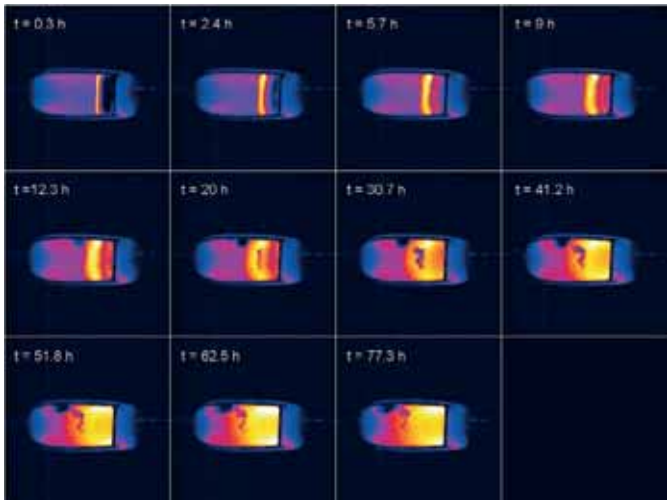
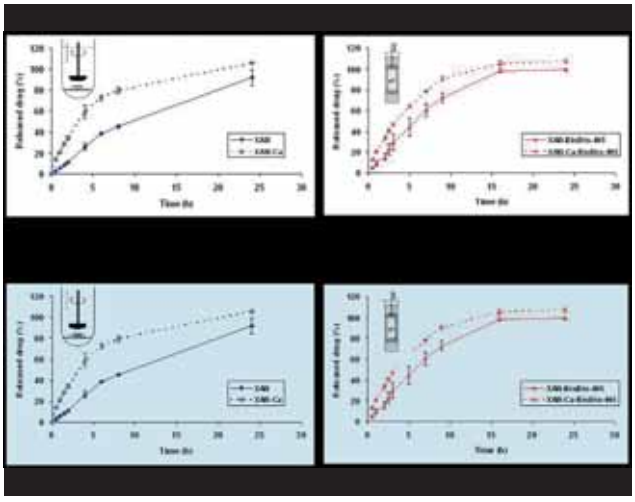
Purpose: The goal was to find thermodynamic criteria that must be satisfied in order to prevent formation of crystalline state of drugs within a confined space (e.g., nanopores of inorganic solid). Similarly, criteria that lead to stabilization of amorphous drug within such pores were investigated. Methods: In the theoretical part, the classical thermodynamics of nucleation is applied to the conditions of a restricted space. The theoretical findings are verified using porous silica as a carrier and nifedipine as a model drug. The amorphicity of the latter is checked using XRD and thermal analysis (DTA, DSC) in combination with BET measurements.

Results: It is shown that there exists a critical pore radius of a host below which the entrapped substance will solidify in an amorphous form. There also exists a critical pore radius below which the entrapped amorphous solid will not be able to crystallize. Specifically, incorporation of NIF into a silica xerogel with an average pore diameter of about 2.5 nm produces and stabilizes its amorphous form.

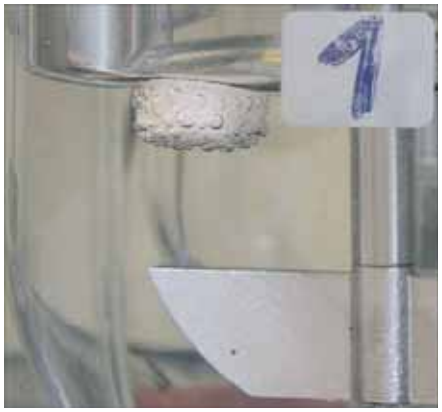
Conclusion: Entrapment of drugs into solid nanoporous carriers could be regarded as a potentially useful and simple method for production and/or stabilization of non-crystalline forms of a wide range of drugs.



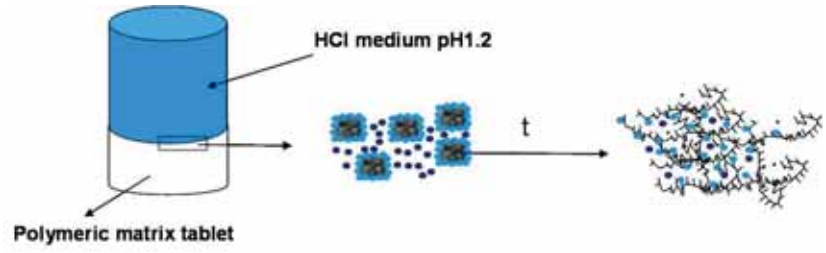
Swelling process of xanthan tablet in HCl solution pH 1.2 followed by magnetic resonance imaging



Release profile of model drug from xantha (XAN) tablets in purified water and in media with $CaCl_2$ to =0,2M (A) using paddle method and (B) using BioDis



Gel structure of the floating tablet with entraped CO_2 bubbles



Schematic representation of HCl pH 1.2 penetration into the matrix tablet and process of polymer chains erosion with time. Blue circles represent molecules of bound and black circles molecules of free water.

J Immunol Methods 2008; 333: 192-196.

A novel reporter gene assay for interferons based on CHO-K1 cells

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Interferons (IFNs) are cytokines playing an important role in the immune response and defence against viruses. They are widely used as biopharmaceuticals. Currently, the anti-viral assay (AVA) is the most commonly used bioassay for determining interferon potency. In the search for rapid and robust but reliable methods, reporter gene assays (RGA) appear to be the most promising approach, therefore we have designed a new reporter cell line, CHO-ISRE-SEAP, suitable for determination of type I interferon potency. Chinese hamster ovary (CHO-K1) cells were stably transfected with secretory alkaline phosphatase (SEAP) gene under the control of interferon stimulated response element (ISRE) promoter. The amount of SEAP in the cell culture medium can be easily measured colorimetrically and has been found to correlate with the amount of IFN added. The new assay is widely applicable for determination of type I IFNs, such as IFN-α, IFN-β and IFN-ω, in research, development of IFN biopharmaceuticals, in batch release, etc. Interestingly, in this assay, IFN-beta shows approximately 6 times higher response than IFN-alpha, which makes it especially appropriate for measuring low levels of IFN-beta. Compared to other known RGAs, the novel CHO-ISRE-SEAP cell line-based RGA appears to have certain advantages with respect to cost and performance.

In: Shoenfeld Y, Gershwin, ME, Meroni P. Autoantibodies. 2nd ed. Boston, MA: Elsevier B. V., 2007; 21-28.

Affinity and avidity of autoantibodies

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The basic knowledge and clinical applications of the detection of affinity/avidity of autoantibodies is relatively scarce. Surprisingly, there have been very few recent studies concerning this topic. While theoretical thermodynamics of antigen-antibody interactions are well known, their practical use in detection or determination of autoantibodies is deficient. Both, affinity as well as specificity for antigens may have important impact for selection of the detection assay. The major problem may be attributed to the lack of suitable measuring methods according to the Law of Mass Action. Selective non-organ specific autoimmune disorders have been chosen to demonstrate the currently existing controversies. Nevertheless, some recent information including new methods and sophisticated technologies give some hope for future usefulness.

Autoimmun Rev 2008; 7: 523-529.

Autoimmune and proinflammatory activity of oxidized immunoglobulins

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AIMS: Oxidation reactions can modify protein activity or specificity. Recently, a novel redox-reactive family of autoantibodies was described, which indicated involvement of altered antibodies (beside altered antigens) into autoimmune reactions. The aim of our study was to determine the binding capacity alterations of electro-oxidized blood donors' IgGs, and to evaluate their effects on released proinflammatory interleukin 6 in HUVEC.
RESULTS: We found out that 1.) Isolated blood donor IgGs bound after electro-oxidation to β2-glycoprotein I, cardiolipin, citrulinated cyclic peptide and protein 3 by enzyme-linked immunosorbent assay, extractable nuclear antigens by counterimmuno-electrophoresis, and cell antigens by indirect immunofluorescence; 2.) Alterations in immunoreactivity of IgGs due to oxidation highly depend on electric current, time of exposure and the presence of antioxidants, 3.) Treatment of HUVEC with oxidized IgGs resulted in changed cell morphology, accompanied by an increase in released interleukin-6.
CONCLUSIONS: Our data suggest repeatable transformation of antibodies present in the blood of healthy persons and patients. Inter-individual differences in chemical stability of antibodies, patient's antioxidant status, and the microenvironmental changes at the cellular level may influence the range of antibody alterations and their involvement in pathophysiological autoimmune processes.

In: Gershwin ME, Shoenfeld Y. Autoimmunity, part A : basic principles and new diagnostic tools, (Annals of the New York Academy of Sciences, v. 1109). Boston, Mass.: Published by Blackwell Pub. on behalf of the New York Academy of Sciences, 2007;1109: 158-166.

Autoimmune reactions after electro-oxidation of IgG from healthy persons: relevance of electric current and antioxidants

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Proteins, including immunoglobulins, can be modified by oxidation. Extensive oxidation of immunoglobulins leads to denaturation and loss of biological activity, while initial steps of oxidation may change their specificity due to chemical alteration of the paratope. Electro-oxidation of the IgG fraction from healthy persons progress to auto-immunoreactivity, as shown for several autoantibodies including anti-β2-glycoprotein I. Changes in immunoreactivity of IgG due to oxidative reactions highly depend on electric current and levels of serum antioxidants. Autoimmune reactions, leading to certain autoimmune diseases, may be partially a consequence of unbalanced anti-oxidative status of an individual.

Immunology 2008; 124: 76-88.

Cysteine protease cathepsin X modulates immune response via activation of [beta][sub]2 integrins

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Cathepsin X is a lysosomal, cysteine dependent carboxypeptidase. Its expression is restricted to cells of the immune system, suggesting a function related to the processes of inflammatory and immune responses. It has been shown to stimulate macrophage antigen-1 (Mac-1) receptor-dependent adhesion and phagocytosis via interaction with integrin beta(2) subunit. Here its potential role in regulating lymphocyte proliferation via Mac-1 and the other beta(2) integrin receptor, lymphocyte function-associated antigen-1 (LFA-1) has been investigated. Cathepsin X has been shown to suppress proliferation of human peripheral blood mononuclear cells, by activation of Mac-1, known as a suppressive factor for lymphocyte proliferation. On the other hand, co-localization of cathepsin X and LFA-1 supports the role of cathepsin X in regulating LFA-1 activity, which enhances lymphocyte proliferation. As shown by fluorescence resonance energy transfer, using U-937 and Jurkat cells transfected with alpha(L)-mCFP and beta(2)-mYFP, recombinant cathepsin X directly activates LFA-1. The activation was confirmed by increased binding of monoclonal antibody 24, recognizing active LFA-1. We demonstrate that cathepsin X is involved in the regulation of two beta(2) integrin receptors, LFA-1 and Mac-1, which exhibit opposing roles in lymphocyte activation.

Autoimmunity 2009; in press

In vitro model of annexin A5 crystallization on natural phospholipid bilayers observed by atomic force microscopy

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Annexin A5 is a potent anticoagulant protein with a thrombomodulatory function. It is frequently mentioned with systemic inflammatory autoimmune disease, which share higher vulnerability to cardiovascular diseases. The protein has the ability to bind to membranes containing negatively charged phospholipids in a calcium-dependent manner. The potent anticoagulant properties of the protein are a consequence of this crystallization, which forms the lattice of annexin A5 over phospholipid surface, blocking its availability for coagulation reactions. Crystallization of annexin A5 has been proven on homogeneous synthetic phospholipids. However, the crystallization of annexin A5 on inhomogeneous, naturally derived phospholipid surfaces, in p3 and p6 crystal form, has now been reported for the first time. Atomic force microscopy was chosen for the observation of the crystallization of annexin A5 on different solid supported phospholipid bilayers. In this study model the optimal results were obtained by using: 0.5 mg/ml lipid vesicles suspension (70% phosphatidylcholine, 30% phosphatidylserine) in HEPES buffer saline with 2 mM $CaCl_2$, large unilamellar vesicles with sizes around 200 nm, 41°C of phase transition temperature and 21 ìg/ml of native annexin A5 in HEPES buffer saline with 2 or 20 mM $CaCl_2$. Results were evaluated by imaging and force measurements. Demonstration that native annexin A5 is able to spontaneously crystallize on naturally derived, inhomogeneous phospholipids is supporting the putative role of annexin A5 crystal structures as possible antithrombotic shield. This in vitro system is probably more appropriate for studying the pathogenetic role of antiphospholipid antibodies.

J Leukoc Biol 2008; 84: 1306-1315.

Maturation of dendritic cells depends on proteolytic cleavage by cathepsin X

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The maturation status of dendritic cells (DCs) is crucial for effective antigen presentation and initiation of the primary immune response. Maturation stimuli cause the adhesion of immature DCs to the extracellular matrix, which is accompanied by recruitment of the CD11b/CD18 [macrophage antigen-1 (Mac-1)] integrin receptor, cytoskeleton reorganization, and podosome formation. Cathepsin X, a cysteine protease expressed in DCs and other APCs, is involved in Mac-1 activation. We have shown that during maturation, cathepsin X translocates to the plasma membrane of maturing DCs, enabling Mac-1 activation and consequently, cell adhesion. In mature DCs, cathepsin X redistributes from the membrane to the perinuclear region, which coincides with the de-adhesion of DCs, formation of cell clusters, and acquisition of the mature phenotype. Inhibition of cathepsin X activity during DC differentiation and maturation resulted in an altered phenotype and function of mature DCs. It reduced surface expression of costimulatory molecules, increased expression of inhibitory Ig-like transcripts 3 and 4 (ILT3 and ILT4), almost completely abolished cytokine production, diminished migration, and reduced the capacity of DCs to stimulate T lymphocytes. These results stress the importance of cathepsin X in regulating DC adhesion, a crucial event for their maturation and T cell activation.

Clin Chem Lab Med 2007; 45: 1366-1372.

Methylprednisolone, cortisol and the cell-mediated immune response in children after ventricular septal defect repair

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Background: This study evaluated the effects of methylprednisolone on cortisol and cell-mediated immune response (T-lymphocytes and HLA-DR+ monocytes) in peripheral blood after open-heart surgery with cardiopulmonary bypass (CPB) for ventricular septal defect.
Methods: A prospective observational study was carried out in a tertiary multidisciplinary neonatal and paediatric intensive care unit. Ten children under 2 years of age received methylprednisolone succinate (30 mg/kg body weight) in CPB priming solutions before the CPB system was connected to the patient during surgery. Before and immediately after and at 24 and 96 h after the operation, T-lymphocytes and HLA-DR+ monocytes were measured by flow cytometry, and methylprednisolone, methylprednisolone succinate and cortisol in blood plasma were assayed by liquid chromatography-mass spectrometry.
Results: The children were divided into groups with normal cardiac index (CI) and low CI. No significant differences in methylprednisolone and cortisol concentrations before and after surgery were found between the two groups. The normal CI group exhibited more than a three-fold decrease in T-lymphocytes 24 h after surgery and a two-fold decrease in HLA-DR+ monocyte fluorescence immediately after surgery.
Conclusions: Children with normal and low CI were differentiated by T-lymphocytes and HLA-DR+ monocytes. Since no differences in methylprednisolone exposure and cortisol plasma levels between the low-CI and normal-CI groups were found, it can be concluded that factors other than methylprednisolone must contribute to differences in the cell-mediated response.

J Cell Sci 2008; 121: 2652-2661.

The role of cathepsin X in the migration and invasiveness of T lymphocytes

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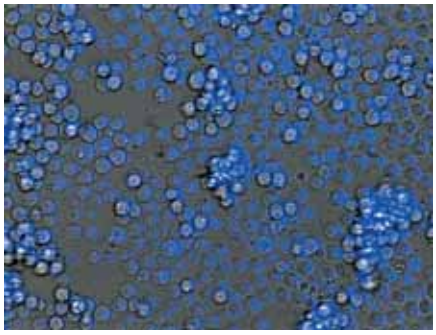
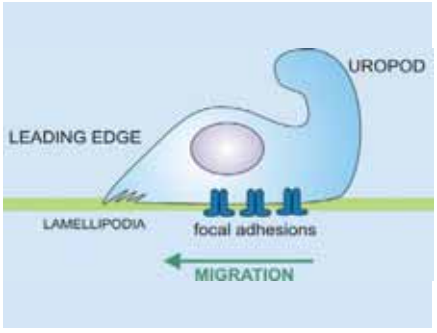
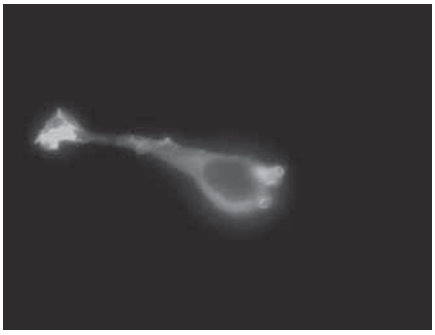
Cathepsin X is a lysosomal cysteine protease exhibiting carboxypeptidase activity. Its expression is high in the cells of immune system and its function has been related to the processes of inflammatory and immune responses. It regulates processes such as adhesion, T lymphocyte activation and phagocytosis through its interaction with beta(2) integrins. To investigate the role of cathepsin X in the migration of T lymphocytes, Jurkat T lymphocytes were stably transfected with a pcDNA3 expression vector containing cathepsin X cDNA. The cathepsin-X-overexpressing T lymphocytes exhibited polarised migration-associated morphology, enhanced migration on 2D and 3D models using intercellular adhesion molecule 1 (ICAM1)- and Matrigel-coated surfaces, and increased homotypic aggregation. The increased invasiveness of cathepsin-X-overexpressing cells does not involve proteolytic degradation of extracellular matrix. Confocal microscopy showed that the active mature form of cathepsin X was colocalised in migrating cells together with lymphocyte-function-associated antigen 1 (LFA-1). The colocalisation was particularly evident at the trailing edge protrusion, the uropod, that has an important role in T lymphocyte migration and cell-cell interactions. We propose that cathepsin X causes cytoskeletal rearrangements and stimulates migration of T lymphocytes by modulating the activity of the O-2 integrin receptor LFA-1.



Jurkat T lymphocyte cell line



During firm adhesion, the combination of integrin signalling and exposure to immobilized chemokines on the apical surface of endothelial cells induces a marked change in the morphology of T lymphocytes. Migration-associated polarization is initiated by polar redistribution of the cell surface receptors and cytoskeletal elements, resulting in the formation of three different morphological and functional compartments: a) the leading edge with one or several lamellipodia rich in F-actin, chemokine receptors and substrate-adhesion molecules; b) the mid-cell region and b) the uropod which is a distinctive region projecting from the trailing edge.



The cathepsin X up-regulated T lymphocytes exhibit polarized migration-associated morphology and enhanced migration through 3D extracellular matrix model.

J Pharmacy Pharm Sci 2008; 11: 67-76.

Evolutionary artificial neural networks as tools for predicting the internal structure of microemulsions

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Purpose: The purpose of this study was to predict microemulsion structures by creating two artificial evolutionary neural networks (ANN) combined with a genetic algorithm. The first ANN would be able to determine the type of microemulsion from the desired composition, and the second to determine the type of microemulsion directly from a differential scanning calorimetry (DSC) curve. Methods: The algorithms and the structures for each ANN were constructed and programmed in C++ computer language. The ANNs had a feed forward structure with one hidden level and were trained using a genetic algorithm. DSC was used to determine the microemulsion type. Results: The ANNs showed very encouraging accuracy in predicting the microemulsion type from its composition and also directly from the DSC curve. The percentage success, calculated over the tested data, was over 90%. This enabled us, with satisfactory accuracy, to construct several pseudoternary diagrams that could facilitate the selection of the microemulsion composition to obtain the optimal desired drug carrier. Conclusions: The ANN constructed here, enhanced with a genetic algorithm, is an effective tool for predicting the type of microemulsion. These findings provide the basis for reducing research time and development cost for characterizing microemulsion properties. Its application would stimulate the further development of such colloidal drug delivery systems, exploit their advantages and, to a certain extent, avoid their disadvantages.

In: Microemulsions: Properties and Applications, Monzer Fanun Ed., Francis Taylor group, 2008; 293-311.

Physicochemical characterization of pharmaceutically applicable microemulsions: Tween40®/Imwitor308®/isopropylmyristate/water

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Microemulsions are promising vehicles for drug delivery. We have studied the structural properties of microemulsions formed from ingredients having potential applicability in pharmacy – surfactant (Tween 40®)/co-surfactant (Imwitor 308®), isopropyl myristate and water. In order to lay the basis for predicting drug release under *in vivo* conditions, where the microemulsion composition varies continuously, the investigation was carried out with a continuous variation of the oil to water ratio over a wide range of surfactant content. Different types and structures of microemulsion were identified by measuring density, surface tension, electrical conductivity, small angle x-ray scattering and differential scanning calorimetry. Analysis of the data permits a qualitative description of the changes in the microstructures appearing in the microemulsion regime of the phase diagram, together with evidence for transitions between them.

J Microencapsulation 2007; 24: 72-81.

Preparation of microcapsules with self-microemulsifying core by a vibrating nozzle method

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Incorporation of drugs in self-microemulsifying systems (SMES) offers several advantages for their delivery, the main one being faster drug dissolution and absorption. Formulation of SMES in solid dosage forms can be difficult and, to date, most SMES are applied in liquid dosage form or soft gelatin capsules. We have explored the incorporation of SMES in microcapsules, which could then be used for formulation of solid dosage forms. An Inotech IE-50 R encapsulator equipped with a concentric nozzle was used to produce alginate microcapsules with a self-microemulsifying core. Retention of the core phase was improved by optimization of encapsulator parameters and modification of the shell forming phase and hardening solution. The mean encapsulation efficiency of final batches was more than 87%, which resulted in 0.07% drug loading. It was demonstrated that production of microcapsules with a self-microemulsifying core is possible and that the process is stable and reproducible.

Eur J Pharm Biopharm 2009; 72: 69-75.

Simultaneous absorption of vitamins C and E from topical ME using reconstructed human epidermis as a skin model

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Antioxidants provide the mainstay for skin protection against free radical damage. The structure of microemulsions (ME), colloidal thermodynamically stable dispersions of water, oil and surfactant, allows the incorporation of both lipophilic (vitamin E) and hydrophilic (vitamin C) antioxidants in the same system. The objective of this work was to investigate the potential of non-thickened (o/w, w/o and gel-like) and thickened (with colloidal silica) ME as carriers for the two vitamins using reconstructed human epidermis (RHE). The amounts of these vitamins accumulated in and permeated across the RHE were determined, together with factors affecting skin deposition and permeation. Notable differences were observed between formulations. The addition of colloidal silica as thickener enhanced the penetration of the antioxidants in RHE and changed the permeation profiles of vitamin C. The location of the antioxidants in the ME and their mobility and affinity for the vehicle appear to be crucial in the case of non-thickened ME. By varying the composition of ME, skin absorption of the two vitamins can be significantly regulated.

Drug delivery, 2007; 14: 235-245.

Stability of Vitamins C and E in Topical Microemulsions for Combined Antioxidant Therapy

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An interesting strategy for protecting skin from excessive exposure to free radicals is to support the skin endogenous antioxidant system. As the balance between different skin antioxidants is very important, a combined therapy using at least two antioxidants is desirable. In the present work, o/w, w/o, and gel-like microemulsions (ME), all composed of the same ingredients, were selected as carrier systems for dermal delivery of vitamins C and E. Gel-like ME was found to offer the best protection for both vitamins, although other ME also significantly increased their stability compared with that solution. In the presence of vitamin C no decrease in vitamin E content occurred. To obtain ME appropriate for dermal use, their viscosity was increased by adding thickening agents. On the basis of visual examination of viscosity and physical stability of thickened systems, several thickeners were selected. The addition of thickener significantly increased the viscosity of ME and changed the behavior of systems from ideal Newtonian to thixotropic. Finally, the stability of both vitamins was examined as a function of thickening agent and of the location of vitamins in the ME. The addition of thickeners changed the stability of at least one vitamin, but the systems generally still protected vitamins better than solutions. It is likely that the changes in internal organization of ME resulting from the addition of thickener, confirmed by thermal analysis and changes in solubility of oxygen in the outer phase, were the most important factors that influenced the stability of vitamins in thickened systems.

AAPS PharmSciTech, 2009

Temperature-Sensitive Microemulsion Gel: An Effective Topical Delivery System for Simultaneous Delivery of Vitamins C and E

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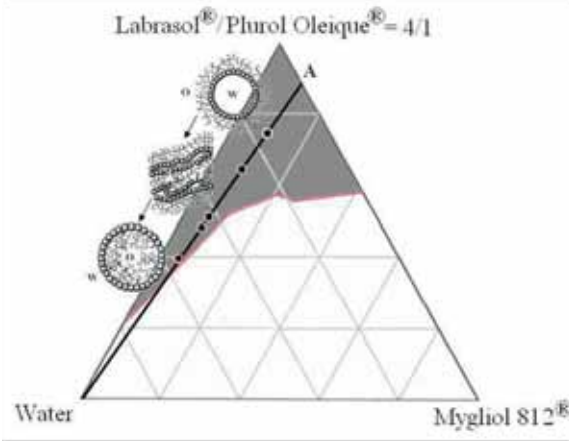
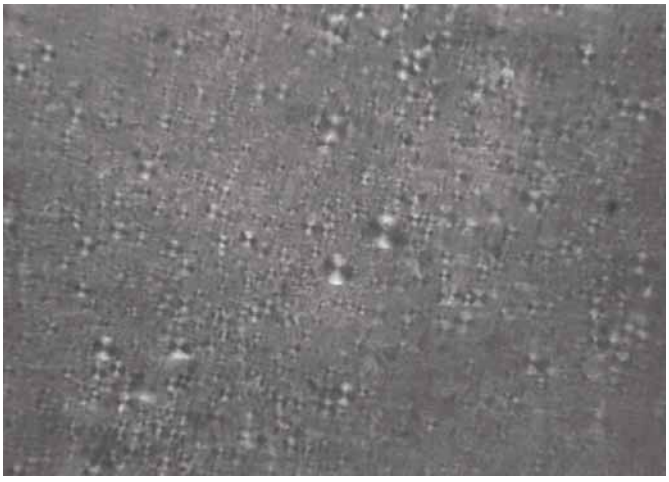
Abstract

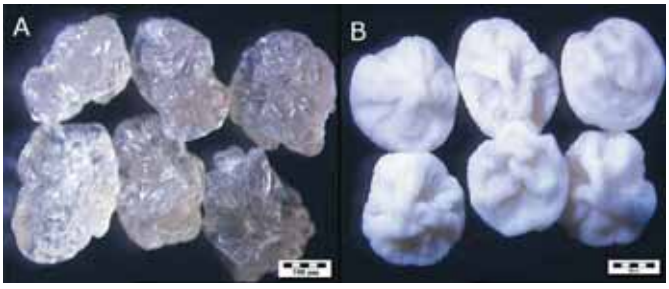
Microemulsions (ME)—nanostructured systems composed of water, oil, and surfactants—have frequently been used in attempts to increase cutaneous drug delivery. The primary objective addressed in this work has been the development of temperature-sensitive microemulsion gel (called gel-like ME), as an effective and safe delivery system suitable for simultaneous topical application of a hydrophilic vitamin C and a lipophilic vitamin E. By changing water content of liquid o/w ME (o/w ME), a gel-like ME with temperature-sensitive rheological properties was formed. The temperature-driven changes in its microstructure were confirmed by rotational rheometry, viscosity measurements, and droplet size determination. The release studies have shown that the vitamins' release at skin temperature from gel-like ME were comparable to those from o/w ME and were much faster and more complete than from o/w ME conventionally thickened with polymer (o/w ME carbomer). According to effectiveness in skin delivery of both vitamins, o/w ME was found the most appropriate, followed by gel-like ME and by o/w ME carbomer, indicating that no simple correlation between vitamins release and skin absorption could be found. The cytotoxicity studies revealed good cell viability after exposure to ME and confirmed all tested microemulsions as nonirritant.



Phase diagram of the microemulsion system with marked structure transitions taking place along the investigated dilution line A; grey area - microemulsions, white area - unstable emulsions.



Ca-pectinate microcapsules with self-microemulsifying core (A) vs. Ca-pectinate microspheres without liquid core (B); samples were dried in fluid bed system



Polarizationsmicrograph of liquid crystal sample with 19,5 % isopropyl myristate, 45,5 % surfactant (lecithin:Tween 80=1:1) and 35,5 % water content with characteristic Maltese cross textures at magnification of 20.

Afr J Biotechnol 2008; 7: 4940-4950.

Bioapplication and activity of fullerenol $C_{60}(OH)_{24}$

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Here we summarize current investigations about a relatively new group of compounds mainly composed of carbon atoms - fullerenes and their derivatives. One of the fundamental characteristics of fullerene is its ability to quench various free radicals, behaving as a "free radical sponge". Moreover, the dual nature of fullerenes to act as either quenchers or generators of cell - damaging ROS could be exploited for development of cytoprotective agents on one side or cytotoxic anti-cancer/antimicrobial agents on the other. In addition, several derivatives have shown immunomodulating, neuroprotective and radioprotective effect. Fullerenes are hydrophobic molecules best dissolved in organic solvents, so potential biomedical applications are restricted by their extremely poor solubility in polar solvents. One of the strategies for improving poor solubility is derivatization. Fullerenol $C_{60}(OH)_{24}$ is a water-soluble derivative of C_{60} with improved chemical properties and potential bioapplicability as a free radical scavenger in biological systems, in oxidative stress induced by xenobiotics or radioactive irradiations. However, solubility of $C_{60}(OH)_{24}$ in water (44 mg/l) is not satisfactory and presents a major drawback in its application as an organo-protector. Improvement of physicochemical characteristics of $C_{60}(OH)_{24}$ and chronic investigations on different animal models as well as in human trials are recommended for establishing its antioxidant effect.

Int J Pharm 2008; 357: 44-54.

Influence of dry granulation on compactibility and capping tendency of macrolide antibiotic formulation

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The effect of dry granulation (roller compaction and slugging) on compactibility and tablet capping tendency in a formulation with macrolide antibiotic and microcrystalline cellulose (MCC) was investigated. Direct tableting of this formulation revealed a pronounced capping tendency. Both dry granulated systems exhibit better compactibility and significant reductions in capping tendency compared to direct tableting. The capping tendency was also reduced through the use of precompression during direct tableting. The main volume reduction mechanism for macrolide antibiotic is fragmentation; this was confirmed by Heckel analysis, the lubricant sensitivity test, and SEM images. The yield pressure (*Py*) of the direct tableting system is lower than the *Py* of dry granulated systems, which indicates the lower plasticity of dry granulated systems. These findings do not explain the lower capping tendency of dry granulated systems compared to direct tableting. The main differentiating bonding mechanism is attributed to long distance intermolecular bonds due to the intense amorphization of macrolide antibiotic that occurs during dry granulation. Amorphization leads to a significant increase in surface free energy and consequently stronger long distance bonding between particles, which can withstand elastic relaxation and therefore reduce the capping problem. Solid bridges probably do not make a notable contribution to the mechanical strength of tablets, due to the brittle nature of the particles and the complex molecular structure of macrolide antibiotic.

J Microencapsul 2008; 25: 90-105.

Shape optimization and characterization of polysaccharide beads prepared by ionotropic gelation

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The shape of drug loaded polysaccharide beads produced by ionotropic gelation has been optimized, with the aim of producing spherical beads suitable for further technological operations, such as coating. The optimization was performed on a model system sodium alginate/theophylline by inclusion of various fillers. Incorporation of excipients markedly influenced the morphological characteristics of the beads. The undesired irregular shape of beads caused by incorporation of the drug could only be improved by incorporating a combination of polycarbophil (PK) and polyvinylpyrrolidone (PVP). The spherical shape of these beads was stabilized mechanically by numerous air bubbles trapped inside the beads, which prevented the collapse of the beads during drying. The optimized method was shown to be applicable to a target system of pectin and an anti-inflammatory drug, LK-423.

Sci Pharm 2008; 76: 77-89.

The influence of selected parameters on the size and shape of alginate beads prepared by ionotropic gelation

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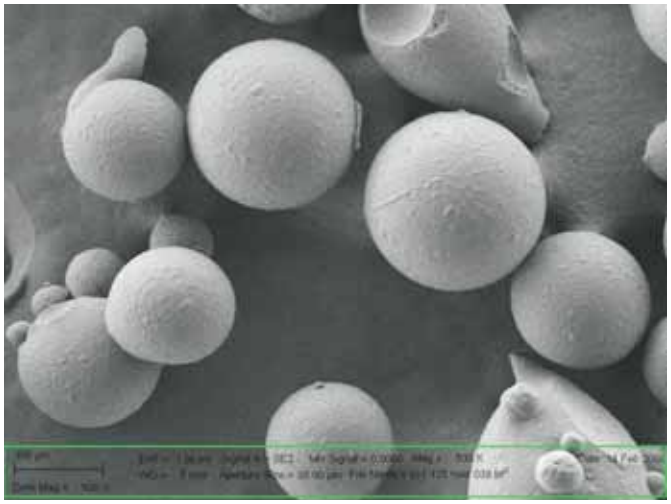
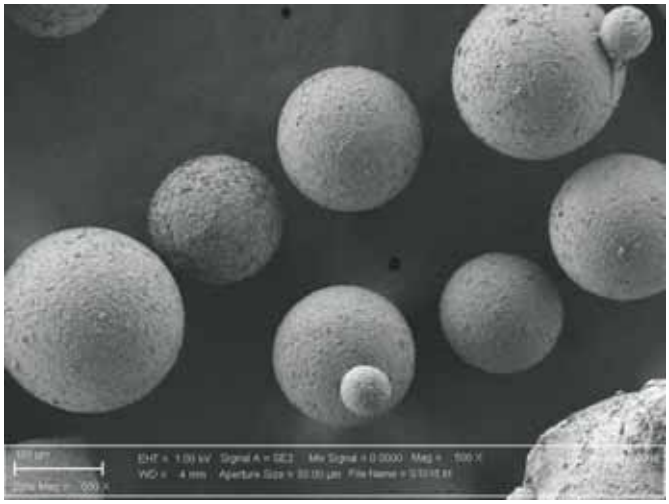
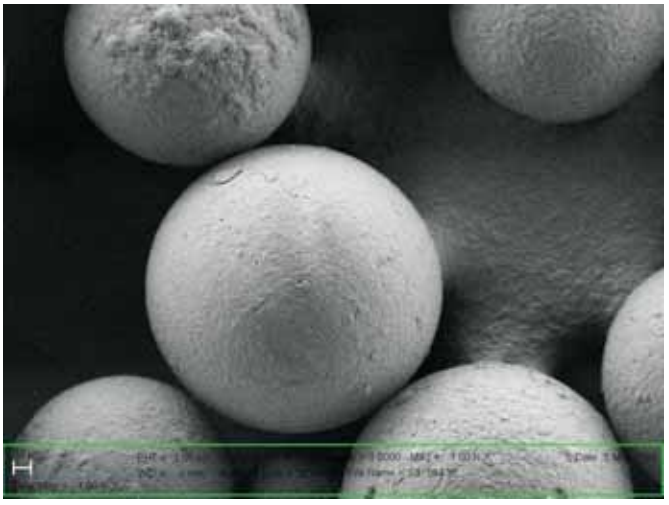
Many bead biopharmaceutical characteristics are dependent on the bead shape. Furthermore, the shape is one of crucial parameters for incorporation of beadsin more complex drug delivery system. Therefore, the aim of this study was to evaluate the influence of various processing parameters such as hardening time, temperature and concentration of calcium chloride solution anddrying conditions on size, shape and morphology of alginate beads prepared by ionotropic gelation method. Theophylline was selected as a model drug. It was found that all studied parameters markedly affected bead form, resembling in most cases to ellipsoid spheres. Their sphericity was estimated three-dimensionally by measuring diameters of frontal and lateral side which were perpendicular to each other. Smaller and more spherical beads were obtained at longer hardening time and higher temperature of calcium chloride solution. The freeze-dried beads were the largest and the most spherical. It was demonstrated that optimization of bead shape as well as size and morphology could be achieved by altering processing parameters.



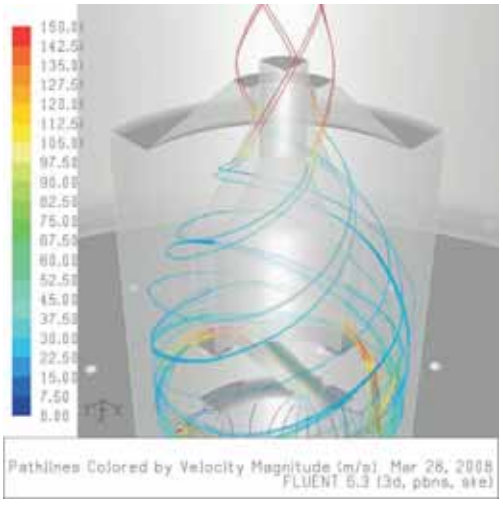
Microparticles produced by spray-congealing.



Microparticles produced by hot-melt technology.



Hot-melt production of microparticles.



Eur J Pharm Biopharm 2008; 69: 23-30.

A novel gene delivery system for stable transfection of thiopurine-S-methyltransferase gene in versatile cell types

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A novel gene transfer system termed artificial viral particles (AVP) containing a plasmid coding for a recombinant fusion protein of enhanced green fluorescent protein (EGFP) with thiopurine-S-methyltransferase (TPMT) was designed for transfection of selected cell lines to establish stable clones which express recombinant EGFP-TPMT protein for further in vitro investigation of toxic effect of thiopurine drugs. AVP based on a complex of the cationic polymer polyethylenimine and anionic liposomes were designed and transfection conditions were adapted in order to transfect the human Jurkat, HepG2 and HEK 293 cell lines with a plasmid coding for EGFP-TPMT. Stably transfected clones were successfully established and expression of recombinant EGFP-TPMT in homogenous cell populations was demonstrated by flow cytometry, fluorescence microscopy and immunoblotting. The level of the expressed protein in stable clones was highest in HEK 293, followed by HepG2 and Jurkat. The enzymatic activity of the TPMT moiety was demonstrated by decreased sensitivity to 6-thioguanine and increased sensitivity to 6-mercaptopurine in HEK 293 cells expressing EGFP-TPMT. Formulation of AVP as transfection vector succeeded in establishing human cell lines stably expressing EGFP–TPMT, thereby proving a successful delivery system and providing an initial step to enable investigation of the role of the clinically important drug metabolizing enzyme TPMT.

J Microencapsul 2009, in press

High celecoxib-loaded nanoparticles prepared by a vibrating nozzle device

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Drug delivery research is resulting in the availability of several enabling technologies for formulating poorly water-soluble compounds. In this study the vibrating nozzle device, originally used for encapsulation of drugs, cells and microorganisms, has been used to formulate nanoparticles (NP) with high loading capacity. Celecoxib was incorporated in NP of polylactic acid (PLA) and poly(lactic-co-glycolic acid) (PLGA) and the influence of polymers, initial drug : polymer ratio and stabilizer concentration on NP size and surface properties, entrapment efficiency, drug loading and *in vitro* release profile were investigated. NP were in the size range of 230 - 270 nm, with a polydispersity index less than 0.25 and a spherical shape. The highest celecoxib loading (13 % w/w) was obtained at initial ratio celecoxib : Resomer RG 502 (PLA/PGA = 50/50) of 1 : 5 and 0.1 % w/w polyvinyl alcohol concentration. Thermal analysis and X-ray diffraction suggested that celecoxib was amorphous or molecularly dispersed in the polymeric matrix. The release profile exhibited an initial burst followed by sustained release. The freeze-dried NP could be completely dispersed on addition of lyoprotectants. The production of NP by the vibrating nozzle device is highly reproducible, time saving, can be performed under aseptic conditions and offers the possibility of scale-up.

Int J Pharm 2008; 359: 220-227.

Influence of nanosized delivery systems with benzyl nicotinate and penetration enhancers on skin oxygenation

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Many novel nanosized delivery systems have been designed for topical application of drugs since theycan overcome the skin barrier and improve drug bioavailability. The increased absorption is often a consequence of a reversibly disrupted barrier function of the skin by the vehicle itself or by specific ingredients that act as penetration enhancers. This paper reports the effects of two nanosized systems (microemulsion and liposomes), in the presence and absence of penetration enhancers (PE), on the topical delivery of a lipophilic drug in vivo and compares that to classical hydrogel formulation. A vasodilator benzyl nicotinate (BN), which increases the blood flow of the skin, was incorporated into the formulations, and skin oxygenation was followed by electron paramagnetic resonance oximetry. It was found that microemulsions and liposomes (with or without PE) accelerate the rate of BN action when compared to hydrogel. However, incorporation of PE in microemulsion also improves the effectiveness of BN action. To understand why PE enhances the action of BN, its effect on the structure of the stratum corneum was investigated in vitro.The increased fluidity of the stratum corneum lipids provides an explanation for the greater penetration of BN into the skin when the drug and PE are together incorporated into the appropriate formulation.

Int J Pharm, 2006; 312: 179-186

Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs.

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Poorlywater-soluble compounds are difficult to develop as drug products using conventional formulation techniques and are frequently abandoned early in discovery. In the present study, the melt emulsification method traditionally used to prepare solid lipid nanoparticles was adapted to produce drug nanosuspensions. The methodwas evaluated in comparison with the well knownsolvent diffusion process for ibuprofen as a model drug. Control of the preparation variables (stabilizers, drug content, homogenization procedure and cooling conditions) allowed formation of nanosuspensions with diameters less than 100 nm. The major advantage of the melt emulsification method over the solvent diffusion method is the avoidance of organic solvents during production, although the mean particle size is slightly greater. The combination of Tween 80 and PVP K25 as stabilizers yields nanosuspensions with the smallest average particle size. The formulation of ibuprofen as a nanosuspension, either in the form of lyophilized powder or granules, was very successful in enhancing dissolution rate, more than 65% of the drug being dissolved in the first 10 min compared to less than 15% of the micronized drug. The increase in in vitro dissolution rate may favourably affect bioavailability and improve safety for the patient by decreasing gastric irritancy.

Article was awarded by Elsevier as the highest cited original article published in International Journal of Pharmarmaceutics in 2006.

J Control Release 2007; 120: 18-26

Targeting cancer cells using PLGA nanoparticles surface modified with monoclonal antibody

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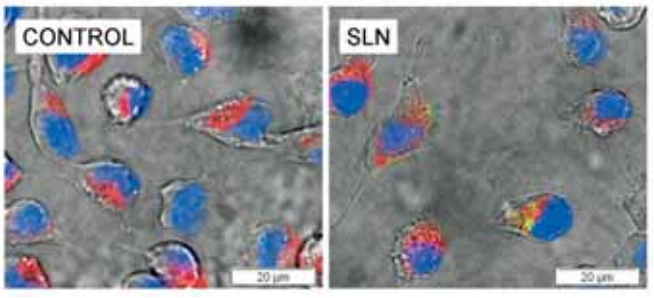
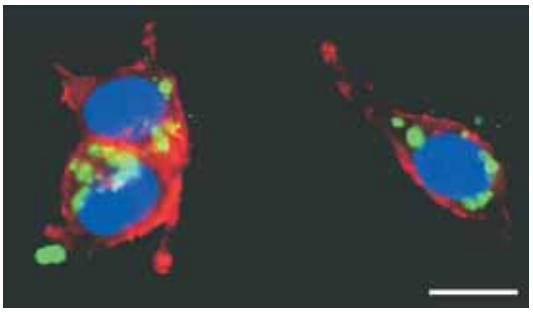
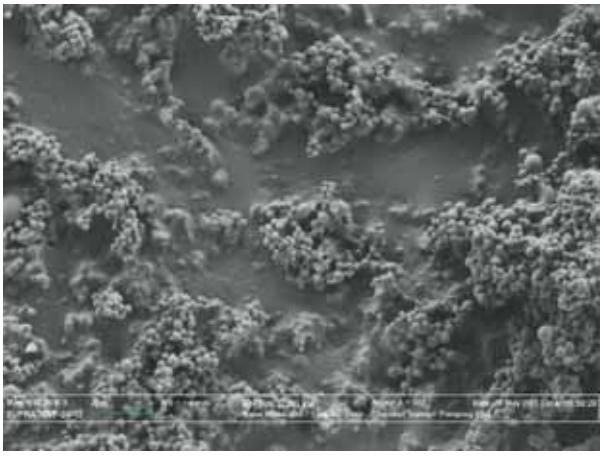
Targeting drugs to their sites of action is still a major challenge in pharmaceutical research. In this study, polylactic-co-glycolic acid (PLGA) immuno-nanoparticles were prepared for targeting invasive epithelial breast tumour cells. Monoclonal antibody (mAb) was used as a homing ligand and was attached to the nanoparticle surface either covalently or non-covalently. The presence of mAb on the nanoparticle surface, its stability and recognition properties were tested. Protein assay, surface plasmon resonance, flow cytometry and fluorescence-immunostaining confirmed the presence of mAb on nanoparticles in both cases. However, a binding assay using cell lysate revealed that the recognition properties were preserved only for nanoparticles with adsorbed mAb. These nanoparticles were more likely to be bound to the targeted cells than non-coated nanoparticles. Both types of nanoparticles entered the target MCF-10A neoT cells in mono-culture. In co-culture of MCF-10A neoT and Caco-2 cells immuno-nanoparticles were localized solely to MCF-10A neoT cells, whereas non-coated nanoparticles were distributed randomly. Immunonanoparticles entered only MCF-10A neoT cells, while non-coated nanoparticles were taken up by both cell types, indicating specific targeting of the immuno-nanoparticles. In conclusion, we demonstrate a method by which mAbs can be bound to nanoparticles without detriment to their targeting ability. Furthermore, the results show the effectiveness of the new carrier system for targeted delivery of small or large active substances into cells or tissues of interest.



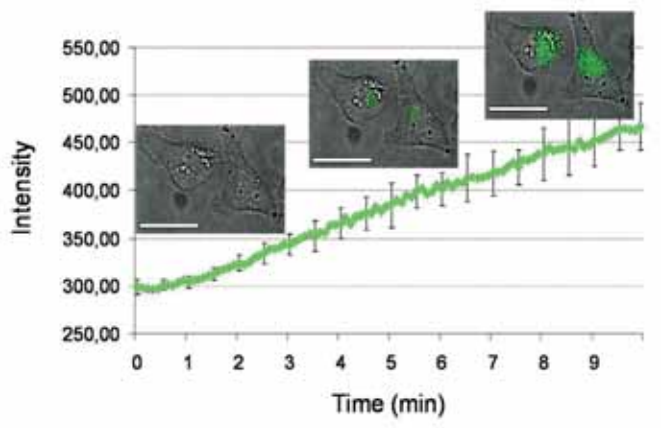
Fluorescent-transmission micrographs of keratinocytes untreated (control) or treated with fluorescent solid lipid nanoparticles (SLN) for 24 h. Mitochondria stained with MitoTracker (red) and nuclei stained with Hoechst 33342 (blue) are embedded by cytoplasmic membrane (transmission image). Pictures were taken using the 60-fold objective on an Olympus IX 81 fluorescence microscope.



Internalization of nanoparticles in HEK293 cells. After 24 hours incubation of cells with fluorescent nanoparticles (seen as green dots), cell nuclei were stained with Hoechst 33342 (blue), and actin fibres with TRITC (red). Pictures were taken using the 100-fold objective on an Olympus IX 81 fluorescence microscope. Bar is 10 m.



Scanning electron micrograph of PLA nanoparticles.



Rapid increase of green fluorescence intensity in the cell cytoplasm, observed after the addition (0 min) of fluorescently-labeled solid lipid nanoparticles. The pictures represent merged transmission and fluorescent images taken at identical camera-focus settings during defined period of time. Bar is 20 m.

Biol Pharm Bull 2009; in press.

Aged garlic extract stimulates P-glycoprotein and multidrug resistance associated protein 2 mediated effluxes

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The growing concomitant consumption of drugs and herbal preparations such as garlic, and the numerous reports about the influence of herbal preparations on intestinal transport, led us to evaluate the influence of aged garlic extract on the transport function and electrophysiological parameters of the small intestinal mucosa. Aged garlic extract induced increase of the absolute value of the transepithelial potential difference and of the short-circuit current in both permeability models tested (rat jejunum, Caco-2 cell monolayers) without affecting transepithelial electrical resistance. It also caused a significant increase of the Pgp and MRP-2 mediated effluxes through rat jejunum of marker substrates Rhodamine 123 and 2,4-dinitrophenyl-S-glutathione, respectively. Rhodamine 123 efflux through the Caco-2 cell monolayers was not altered by aged garlic extract, whereas the efflux of 2,4-dinitrophenyl-Sglutathione increased significantly. So altered activity of the important transport proteins could significantly change the pharmacokinetic properties of conventional medicines taken concomitantly with aged garlic extract.

Food Chem 2008; 110: 691-696.

Distribution of selenium and phenolics in buckwheat plants grown from seeds soaked in Se solution and under different levels of UV-B radiation

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Seeds of common buckwheat (Fagopyrum esculentum) were soaked in water, sodium selenate (5, 10 or 20 mg Se-VI/L), or sodium selenite (10 or 20 mg Se-IV/L) solutions. Plants grown from soaked seeds were exposed to reduced UV-B radiation, ambient, or enhanced UV-B. The mass fraction of selenium in leaves was much higher in plants obtained from seeds soaked with selenate (up to 185 ng/g) in comparison to selenite (up to 103 ng/g). In plants obtained from seeds soaked in water, regardless of UV-B levels, the highest concentration of selenium was found in leaves, where the values were between 45 and 66 ng Se/g. In buckwheat leaves 44.5-63.6 mg/100 g d.m. of fagopyrin was found, and in stems 14.3-26.4 mg/100 g d.m.; here no influence of seed soaking solution or UV-B exposure was found. The content of total flavonoids in leaves was 7.8-15.9% and in stems 1.4-4.1%.

Food Chem 2008; 109: 293-298.

Salicylaldehyde is a characteristic aroma component of buckwheat groats

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Salicylaldehyde (2-hydroxybenzaldehyde) was identified as a characteristic component of buckwheat groats aroma by a sensory analysis guided fractionation of the extract. The extract with the strongest odour was prepared by petroleum ether extraction of water soaked groats. This extract was further extracted with sodium hydrogen carbonate solution and purified by a preparative layer chromatography and identified by NMR, MS and IR spectroscopy. A capillary electrophoresis method was developed and used to determine salicylaldehyde content in buckwheat groats and flour samples. Traditionally dehulled buckwheat grain, which had the strongest odour, contained the highest concentration (1.6 ppm) of salicylaldehyde with an odour activity value (OAV) of 216.

Phytother Res 2008, Epub ahead of print.

Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition

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Lipids are important components in human nutrition; however, their increased intake contributes to the development of obesity and can lead to multiple long-term complications. Pancreatic lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) is a key enzyme for the absorption of dietary triglycerides. Interference with fat hydrolysis results in the reduced utilization of ingested lipids, therefore inhibition of lipases decreases fat absorption. Extracts from 106 species of medicinal plants, vegetables and fruits were screened for potential lipase inhibitory activity. p-Nitrophenylpalmitate and 5-bromo-4-chloro-3-indoxylpalmitate were used as substrates in an in vitro test with crude porcine pancreatic lipase. Bearberry (Arctostaphylos uva-ursi), garden pea (Pisum sativum), Norway spruce (Picea abies) and large-leaved lime (Tilia platyphyllos) extracts were the most active. Additionally, the activity of selected extracts with removed polyphenols was measured. Extracts of bearberry, garden pea and large-leaved lime are a promising source for developing functional foods or isolating active compounds.

Common houseleek, Sempervivum tectorum L.

Lady's mantle, Alchemilla vulgaris L.

Caraway, Carum carvi L.

Cowslip, Primula veris L.

Biol Pharm Bull 2009; in press.

Antioxidant levels in the pig urinary bladder: distribution within the bladder wall and in the urothelium derived from different bladder regions

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This study was designed to determine the antioxidant levels in the urinary bladder wall layers as well as urothelium derived from different bladder regions. Samples of the urothelium, lamina propria, muscularis, and serosa were prepared from the pig's urinary bladder body, while samples used for regional mapping of the urothelium were prepared from trigone, ventral and dorsal middle bladder body, and apex region. Activities of superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase were determined. Concentrations of ascorbic acid and glutathione were also measured. Antioxidant activities, i.e. concentrations of superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione, were shown to be highest in the urothelium and progressively lower towards the serosa. Regional mapping of the urothelium singled out apex as the region with the lowest antioxidant activities, i.e. concentrations of glutathione peroxidase, ascorbic acid, and glutathione. The fact that antioxidants are concentrated in the urothelium implies that urothelium functions as a barrier against reactive species. The urothelium derived from the apex is the region with the lowest antioxidant levels and is therefore probably the region most liable to development of oxidative damage.

J Drug Deliv Sci Technol 2007; 17: 173-176.

Bioavailability of metoclopramide from a new chewing gum device

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3 Institutes of Biologica Research, Calleriio Foundation-Onlus, Trieste, Italy
4 University of Trieste, Department of Pharmaceutical Sciences, Trieste, Italy

Metoclopramide is widely used in the treatment of nausea. The development of metoclopramide as medicated chewing gum has potential advantages in terms of patient compliance, fast onset of effect and improved bioavailability. In this study, bioavailability of metoclopramide from a new chewing gum device, 3TabGum, was evaluated in healthy volunteers. Medicated chewing gum was well tolerated in all subjects. Compared with immediate release tablets, AUC following administration of chewing gum was higher (224.9 vs. 166.5 ng h/mL), while t(max) was smaller (1.38 vs. 1.88 h), suggesting improved bioavailability and rapid onset of drug absorption. However, absorption rate was lower, as evidenced by lower C-max (17.2 vs. 20.8 ng/mL). Terminal half-life was prolonged (11.58 vs. 5.35 h), implying that initial fast release of metoclopramide is followed by much slower release of the remaining smaller portion of the drug. These results indicate that chewing gum is a promising alternative to current metoclopramide formulations.

Br J Clin Pharmacol 2009, in press

Effects of UGT1A1*28 polymorphism on raloxifene pharmacokinetics and pharmacodynamics

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Aims Raloxifene concentrations were reported to approximately correlate with serum bilirubin levels. Bilirubin is a typical UGT1A1 substrate. Based on these facts, we postulated a hypothesis that UGT1A1 is the key enzyme for metabolic clearance of raloxifene and that the common UGT1A1*28 polymorphism significantly contributes to the large pharmacokinetic variability of raloxifene. Methods Serum samples from postmenopausal osteoporotic patients treated with raloxifene were assayed for the concentrations of raloxifene and its glucuronides by LC-MS-MS. The same samples were also genotyped for the presence of UGT1A1*28 polymorphism by the SSCP method. The pharmacodynamic effect was evaluated by measuring the change in bone mineral density (BMD) in femoral neck, hip and lumbar spine after 12 months of raloxifene therapy Results Patients homozygous for the *28 allele showed a significantly, two-fold higher raloxifene glucuronide concentrations compared to the hetero- and homozygotes for the wild-type allele: (558 115) nmol/L compared to (295 43) nmol/L, respectively, p=0.012. This indicates a higher raloxifene exposure in the *28/*28 group. Consequently, a significantly greater increase in hip BMD was observed in subjects homozygous for the *28 allele compared to the group carrying at leastone copy of the wild-type allele: (4.4 2.4) % compared to (0.3 1.4) %, p=0.035. Conclusions In this study it was shown that a relatively commonUGT1A1*28 polymorphism may considerably influence raloxifene pharmacokinetics and pharmacodynamics. Underlying mechanisms and clinical implications of our findings are also discussed.

Pharmazie 2009, in press

Enhanced permeability of the urinary bladder wall: the role of polymer charge

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The urothelium is usually impermeable to substances present in the urine. In the current work the possibility of using different absorption enhancers in the development of intravesical drug delivery systems was explored. To establish the role of the polymer charge on its ability to improve bladder wall permeability, cationic poly-L-arginine, anionic NaCMC and alginate as well as nonionic HPC and HPMC were tested. The permeability experiments were performed on isolated pig urinary bladders. We established that the charge of the polymer affects its ability to enhance the permeability of the urinary bladder wall, but to a limited extent. Positively charged polymers were the most promising absorption enhancers for the urinary bladder wall. In order to significantly enhance the permeability of the bladder wall, higher concentrations of poly-L-arginine were needed compared to chitosan. Moreover, chitosan reached the plateau of its absorption enhancement effect after 60 min, while poly-L-arginine increased the permeability continuously over 90 min. In contrast to polycarbophil, two other anionic polymers, NaCMC and alginate, did not significantly enhance the permeation of pipemidic acid into the tissue. Interactions between the polymers and the drug might prevail over the potential effect of NaCMC and alginate on tissue permeability. Furthermore, for the nonionic polymers HPMC and HPC an insignificant influence on bladder wall permeability was determined. Therefore, the selection of absorption enhancers for intravesical drug delivery systems is limited and cannot be done only on the basis of polymer charge.

Eur J Pharm Biopharm 2007; 66: 281-285.

Fluorescein transport properties across artificial lipid membranes, Caco-2 cell monolayers and rat jejunum

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Membrane transport characteristics of a paracellular permeability marker fluorescein were evaluated using artificial membrane, Caco-2 cell monolayers and rat jejunum, all mounted in side-by-side diffusion cells. Modified Ringer buffers with varied pH values were applied as incubation salines on both sides of artificial membrane, cell culture monolayers or rat jejunum. Passive transport according to pH partition theory was determined using all three permeability models. In addition to that, active transport of fluorescein in the M–S (mucosal-to-serosal) direction through rat jejunum was observed. The highest M–S P_{app} values regarding the active transport through the rat jejunum were observed in incubation saline with pH 6.5. Fluorescein transport through the rat jejunum was inhibited by DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid) and α-CHC (α-cyano-4-hydroxycinnamic acid). Thus, we assume that two pH-dependent influx transporters could be involved in the fluorescein membrane transport through the intestinal (jejunal) epithelium. One is very likely an MCT (monocarboxylic acid cotransporter) isoform, inhibited by specific MCT inhibitor α-CHC, while the involvement of the second one with overlapping substrate/inhibitor specificities (most probably a member of the organic anion-transporting polypeptide family, inhibited at least partially by DIDS) could not be excluded.

Pharm Res 2009; in press

Gastric Emptying of Pellets under Fasting Conditions: A Mathematical Model

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Purpose. To develop a mathematical model that would adequately describe human gastric emptying of pellets under fasting conditions of healthy subjects.

Methods. Scintigraphic profiles representing the gastric emptying of pellets were obtained from the literature. Altogether 19 individual and three mean scintigraphic profiles were collected. Three mathematical models namely; the lag-time exponential (two parameters), the Weibull (two parameters), and the double Weibull (five parameters) model were proposed and fitted to the gastric emptying profiles.

Results. Different patterns of gastric emptying (immediate and rapid, delayed but rapid, delayed and slow, and interruptive emptying) were observed, with the emptying time varied from approximately 15 min to more than 3 h. The best model for fitting to the individual profiles was the double Weibull model. This model also provided an insight into the mechanism of interruptive emptying of pellets, observed for some patients. In addition, mean gastric emptying of pellets was calculated using the Weibull model.

Conclusions. Mean gastric emptying of pellets was adequately described by the Weibull model (η= 61.9 min, β=0.895), which could be applied in the design of in vitro dissolution experiments for pellet formulations with pH dependent dissolution.

Drug Dev Ind Pharm 2008; 34: 215-220.

Heparin decreases permeability of pig urinary bladder wall preliminarily enhanced by chitosan

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Chitosan significantly increases the permeability of the isolated pig urinary bladder wall by causing urothelial desquamation, the extent of which depends also on the concentration of the polymer. By desquamation permeability barriers of the urothelium are removed. To gain additional insight into the mechanism by which chitosan acts an absorption enhancer into urinary bladder mucosa, we evaluated the influence of a polysaccharide heparin on the permeability of isolated pig urinary bladder wall preliminarily treated with chitosan. Moreover, we aimed to establish whether the effect of heparin depends on its concentration and on the degree of urothelial desquamation caused by chitosan. In the permeability studies performed by the use of diffusion cells, transport of a model drug, pipemidic acid, into the isolated pig urinary bladder wall was determined. Heparin did not have a significant effect on the permeability of the intact urothelium. When applied to the urinary bladder wall, whose permeability was preliminarily enhanced by 0.005% or 0.001% w/v chitosan, heparin decreased the permeation of pipemidic acid into the bladder wall to a level not significantly different from the intact tissue. However, the effect of heparin was not significant at the highest concentration of chitosan tested, where the damage to the urothelium was much more intense compared with that found at lower concentrations of the polymer. The formation of complexes between pipemidic acid and heparin cannot be excluded completely, but it seems that they are not the main reason for the decreased permeation of pipemidic acid in the presence of heparin. By application on the urothelium, damaged by chitosan, heparin is supposed to form a layer on the surface of the urothelium that prevents the transport of the model drug into the bladder wall. In this way heparin probably restores the impermeability properties of the urinary bladder wall to a degree dependent on the urothelial damage.

Int J Pharm 2009, in press.

Influence of luminal monosaccharides on secretion of glutathione conjugates from rat small intestine in vitro

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Intestinal efflux transporters can significantly reduce the absorption of the drug after peroral application. In this work we studied secretion of glutathione conjugates triggered by glucose at the luminal side of the intestine. Glucose stimulated secretion of DNPSG, NEMSG and CDNB. We used some different monosaccharides and determined that glucose, galactose and α-methylglucopyranoside trigger the secretion, while mannitol and fructose do not. We concluded that interaction with SGLT transporter is the key process necessary for this triggering. To determine which of possible glutathione conjugate transporters (MRP2, MRP4, BCRP or RLIP76) is responsible for the secretion of glutathione conjugates, we used benzbromarone, a MRP inhibitor, and sulfanitran and furosemide, two allosteric MRP2 activators. Benzbromarone inhibited glucose stimulated DNPSG secretion, while allosteric activators additionally increased the secretion. We concluded that MRP2 transporter is related to glucose stimulated DNPSG secretion. Regarding the work of Kubitz et al. we tested the effect of changed medium osmolarity on DNPSG transport and determined that hypoosmolar conditions trigger secretion of DNPSG. These findings suggest that intestinal MRP2 activity has no basal level, but can be stimulated by hypoosmolarity and SGLT transport.

J Agric Food Chem 2007; 55: 9733-9738.

Kinetics of abamectin disposition in blood plasma and milk of lactating dairy sheep and suckling lambs

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Abamectin (ABM) has been used worldwide as an anthelmintic drug in veterinary medicine and as an agricultural pesticide. Its pharmacokinetics and permeation into milk was evaluated in dairy sheep after subcutaneous administration. ABM elimination half-lives and mean residence times were 1.7 and 3.7 days for blood plasma and 1.9 and 3.8 days for milk, respectively. The ABM milk to plasma concentration ratio (0.89) primarily depends on milk fat content. Transfer of ABM residues to suckling lambs was evaluated by determination of ABM concentration time courses in lambs' plasma. Mean maximal concentration in lambs was 1.6 mu g L-1 at 3.3 days, and elimination half-life was 2.7 days. In ewes' plasma and milk, ABM was detected up to 23 days. Because of different pharmacokinetics, ABM exposure in lambs was almost 10% of the exposure in ewes, although the amount excreted in milk was only 1.0% of the dose.

Vet Parasitol 2008; 154: 129-136.

Linearity of eprinomectin pharmacokinetics in lactating dairy sheep following pour-on administration: Excretion in milk and exposure of suckling lambs

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Pharmacokinetics of eprinomectin (EPR) were studied in blood plasma and milk in two groups of six Istrian Pramenka dairy sheep and their suckling lambs following pour-on administration of EPR to ewes at dose levels of 0.5 and 1 mg/kg. Maximum concentration in plasma was 2.22 and 5.25 mu g/l, and AUC was 13.6 and 33.7 mu g day/l for the 0.5 and 1.0 mg/kg dose, respectively. These results indicate that drug exposure with a dose of 0.5 mg/kg, which is commonly used in cattle, may be subtherapeutic. The concentration time course in milk paralleled plasma concentrations. In the dose range studied, linear pharmacokinetics of EPR were demonstrated. Milk-to-plasma AUC ratio was 0.79 +/- 0.12 and 1.12 +/- 0.43; the fraction of dose recovered in milk was 0.037 +/- 0.011 and 0.058 +/- 0.027% for the low and high dose, respectively. Maximum residual levels in milk were below the maximum acceptable level of 20 mu g/kg; however, EPR was detected in all samples investigated. Despite low permeability in milk, AUC in plasma of suckling lambs was between 20 and 30% of the AUC in plasma of ewes.

Chem Biol Drug Des 2008; 72: 217-224.

Membrane permeability of acylated cystatin depends on the fatty acyl chain length

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Hydrophobization of proteins, such as chemical acylation, has been recognized as an efficient method for improving their membrane permeability. In this research, chicken cystatin, a model protein inhibitor of cysteine proteinases, was acylated with fatty acyl residues of 6-18 carbon atoms. The chemical modification was performed using fatty acyl chloride dispersion in aqueous acetonitrile solution. The reaction products were analyzed by capillary electrophoresis, SDS-PAGE and isoelectric focusing. In vitro inhibitory activity was determined by N-benzoyl-D,L-arginine-beta-naphthylamide assay and membrane permeability properties of non-acylated and acylated cystatin by measuring its efficiency to inhibit intracellular cathepsin B in MCF-10A neo T cells. The experiments showed that acylated cystatin quickly internalized into the cells and effectively inhibited cathepsin B. In contrast, non-acylated cystatin did not cause inhibition as it was unable to enter the cell. The permeability enhancement effect was shown to depend on the length of the attached fatty acyl chain as the strongest inhibition was caused by cystatin acylated with stearoyl chloride. In addition, chemical modification did not influence the protein's immunogenicity.

Patent application SI: P-200800189. Ljubljana: The Slovenian Intellectual Property Office, 2008, 9 pages.

Peristaltic movement simulating stirring device for dissolution testing

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The present invention relates to peristaltic movement simulating stirring device for dissolution testing and relates to the field of dissolution testing and particular to dissolution testing device which simulates the movement in gastro-intestinal tract. A peristaltic movement simulating stirring device for dissolution testing is characterized by a suitable amount of beads added into the stirring vessel comprising a stirring bar and a medium simulating gastrointestinal environment. Beads are added in the amount to achieve the minimum amount which covers the bottom of the vessel in one layer to maximum amount which represents the height of about 400% of the stirring bar diameter. The density of the beads is from 1.1 to 5 g/cm^3 and the diameter of the beads is 0.2 – 2 mm. The beads are of spherical shape and made of glass, Teflon™ or other inert material which does not release any substances or particles and prevents the adsorption of drug substances on the surface of the beads. The rotational speed of the stirring bar is from 5 rpm to 150 rpm.

Phytomedicine 2008; 15: 547-554.

Pharmacokinetics and immunomodulatory effects of phytotherapeutic lozenges (bonbons) with Echinacea purpurea extract

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The relative bioavailability of the major alkamides, dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides, from Echinacea purpurea phytotherapeutic lozenges at three different dose levels (0.07, 0.21 and 0.9 mg) was evaluated in a pharmacokinetic study in humans and the possible effects on the immunological system were measured. Alkamides were found to be rapidly absorbed and measurable in plasma 10 min after administration of 0.21 and 0.9 mg lozenges and remained detectable for 3 h for the 0.21 mg lozenges and for more then 3 h for the 0.9 mg lozenges; 0.07 mg lozenges were measurable 20 min after administration and remained detectable for only 2 h after the administration. A significant dose-independent down-regulation of the pro-inflammatory cytokines IL-12p70, IL-8, IL-6, IL-10 and TNF was observed 24 h after oral administration. The results of non-compartmental pharmacokinetic analysis revealed that a C-max of (0.65 +/- 0.41 ng/ml) was reached at 32 min with the 0.07 mg lozenges, (1.00 +/- 0.21 ng/ml) at 25 min with the 0.21 mg lozenges and (8.88 +/- 5.89 ng/ml) at 19 with the 0.9 mg lozenges. As evidenced by the dose-exposure relationship, no significant departure from dose proportionality was observed, indicating linearity in pharmacokinetics. To get a further insight in pharmacokinetics of dodeca-2E,4E,8Z,10E/Z-tetraenoic isobutylamides a compartmental population pharmacokinetic model was developed applying mixed effect modelling procedure. The results demonstrate that within the dose range studied pharmacokinetics of dodeca-2E,4E,8Z,10E/Z-tetraenoic isobutylamides are linear and that absorption is very rapid (t(1/2) = 6 min) with apparently no lag time, thus indicating the possibility that a fraction of the drug is absorbed through the oral mucosa.

Ther Drug Monit 2007; 29: 781-788.

Population pharmacokinetic model of carbamazepine derived from routine therapeutic drug monitoring data

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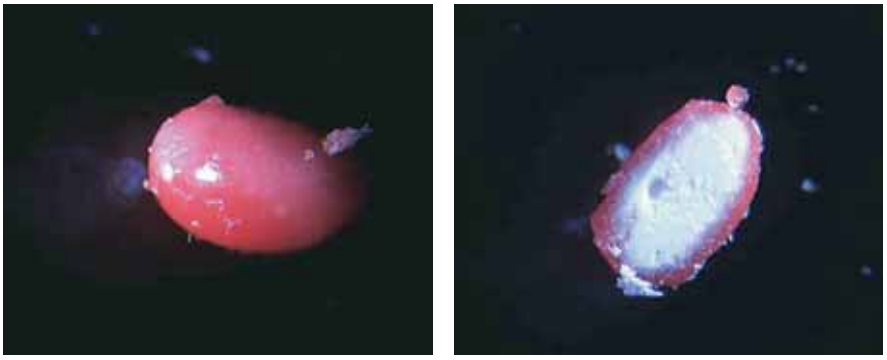
The aim of the present study was to develop a population pharmacokinetic model of carbamazepine from routine therapeutic drug monitoring data. Steady-state carbamazepine plasma concentrations determined by homogenous enzyme immunoassay technique, dosing history including cotherapy, schedule of blood sampling, and patients' demographic characteristics were collected retrospectively from patients' chart histories. A one-compartment model was fitted to the data using nonlinear mixed effects modeling. The influence of weight, age, gender, smoking, allergy, carbamazepine daily dose, and cotherapy on clearance (CL/F) was evaluated. Additionally, bioavailability of controlled-release relative to immediate-release tablets was assessed. Two hundred sixty-five patients (423 concentrations) were used to develop a population pharmacokinetic model. The population estimate of CL/F from the base model was 5.14 L/h with interindividual variability of 50.20%. Patients' gender, age, smoking, allergy, cotherapy with lamotrigine and benzodiazepines had no effect on CUE Patient weight (WT), daily carbamazepine dose (DCBZ), daily dose of phenobarbitone (DPB) and valproic acid (VPA), when its daily dose exceeded 750 mg, significantly influenced CL/F and were included in the final model:
CL/F [L/h] = 5.35 (DCBZ [mg/da/kg]/15)(0.591) (1 + 0.414 (DPB [mg/day/kg]/2)

(WT [kg]/70)(0.564) 1.18(VPA)
where VPA is 1 if dose is greater than 750 mg or 0 otherwise. No difference in bioavailability of carbamazepine between controlled and immediate-release tablets was detected. The model predictions in the validation set had no bias and satisfactory precision. The model can be used for estimation of carbamazepine CL/F in individual patients in the postautoinduction phase and for selection of optimum dosing regimen in routine patient care.



Microcapsules (magnified 32000)

Tissue sample for permeability studies

HPLC / MS / MS (Varian 1200L)

Tetrahedron 2009; 50: 567-569.

A new glucosamine-containing amphiphilic spin probe

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A new, nonionic spin probe for investigation the extracellular matrix close to the cell membrtane by ESR spectroscopy has been synthesized and characterized. A pyrrolidine type nitroxide spin label has been introduced to the third possition of a nonionic polar head (glucosamine) bonded to a lipophilic stearic acid acyl chain anchor. The compound is soluble in polar solvents such as ethanol and chloroform, but sparingly soluble in water.

Tetrahedron Lett; 2008, 49, 225-228.

A pentacyclic condensation product from 2,4-dimethyl-7-nitro-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-2-carboxylic acid

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2,4-Dimethyl-7-nitro-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylic acid on preparation of a mixed anhydride, followed by reduction with sodium borohydride, affords 5,7a,13,13b-tetramethyl-2,10-dinitro-5a,7,7a,13,13a,13b-hexahydro-5*H*-[1,4]benzoxazino[30,20:4,5]pyrano[3,2-b][1,4]benzoxazine (**3**), the structure of which was established unambiguously by X-ray analysis.

Tetrahedron Lett 2007; 48: 4403-4405.

An improved total synthesis of UDP-N-acetyl-muramic acid

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Biochemical testing of novel inhibitors of Mur ligases reguires several commercially unavailable and structurally complex substrates. We described a modified synthetic strategy for the total chemical synthesis of the MurC ligase substrate UDP-*N*-acetyl-muramic acid which includes several improvements over published methods, especially with regards to purification procedures. The synthetic strategy is applicable for the synthesis of further Mur ligase substrates.

Tetrahedron Lett 2009; 50: 564-566.

Microwave assisted synthesis of amphiphilic spin probes

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New amphiphilic spin probes have been synthesized. The key reaction is based on microwave-assistant epoxide ring opening with amines as nucleophiles using calcium trifluoromethanesulfonate as a catalyst. High yields, in short reaction times, were obained without any detectable nitroxide decomposition.

Tetrahedron 2007; 63: 141-147.

Microwave-assisted synthesis of hydroxyethylamine dipeptide isosteres

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Microwave irradiation with calcium trifluoromethanesulfonate as a catalyst enable epoxide opening with protected amino acids in high yields and with very short reaction times. Using this improved method, a series of hydroxyethyleneamine dipeptide isosteres was synthesized.

Pharmazie 2008; 63: 102–106.

Optimization of UDP-N-acetylmuramic acid synthesis

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UDP-N-acetylmuramic acid (UDP-MurNAc) is a substrate of MurC, an important enzyme in the intracellular pathway of bacterial peptidoglycan biosynthesis. Various approaches towards preparation of UDP-MurNAc have been published but these synthetic preparations were shown to include many problematic steps. An optimization study with the focus on muramyl phosphate and UMP-morpholidate coupling was performed, resulting in a synthetic procedure enabling robust and easily reproducible production on a multi-gram scale

J Pept Sci 2008, 14, 946-953.

Peptides and pseudopeptides incorporating D-Phe-Pro-Arg and Arg-Gly-Asp lead sequences as potential antithrombotic agents

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Peptide leads *D*-Phe–Pro–Arg for thrombin inhibition and Arg–Gly–Asp for antagonistic activity on fibrinogen receptor were combined in one molecule in order to produce compounds capable of acting both as thrombin inhibitors and as fibrinogen receptor antagonists. Peptide conjugate **7** possessing both leads joined by a tetraglycine linker as well as tripeptides and peptidomimetics with highly overlapped *D*-Phe–Pro–Arg and Arg–Gly–Asp pharmacophore groups were prepared. Conjugate **7** was found to possess antagonistic activity on fibrinogen receptor, but was unexpectedly inactive as thrombin inhibitor. Compound **9** comprising of highly integrated *D*-Phe–Pro–Arg and Arg–Gly–Asp pharmacophore groups was found to possess a moderate but well balanced thrombin inhibitory and fibrinogen receptor antagonistic activity.

Synlett 2007: 1257-1258.

Preparation of amidines by amidoxime reduction with potassium formate

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We prepared several amidoximes by reacting the starting nitrile with hydroxylamine hydrochloride in the presence of a base and employed two approaches to reduce them to amidines, one under slightly basic and acidic conditions, the second by acylating the amidoximes and reducing them under acidic conditions. Reduction of amidoximes with HCOOK in acetic acid proceeded slowly and was complete in 24 hours (data notshown). When using HCOOK in MeOH, however, the reaction was incomplete even after 24 hours, indicating that the acidic medium accelerates the reduction.

Synth comm 2008; 38: 3422-3438.

Preparation of saccharin derivatives of amino acids as potential peptidomimetic building blocks

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We present a useful route for the preparation of saccharin derivatives of amino acids. Sixteen new compounds, saccharin-derived amino acids, were synthesized, all of them bearing additional functional groups either at the 5- or 6-position of the saccharin skeleton, thus rendering the compounds more amenable to functionalization.

Helv Chim Acta 2008; 91: 654-664.

Ring Opening of 2-Benzylamino-2H-1,4-Benzoxazin-3(4H)-ones and 2-Bromo-2H-1,4-benzoxazin-3(4H)-ones

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Substituted 2-(benzylamino)-2*H*-1,4-benzoxazin-3(4*H*)-ones are unstable under alkaline and acidic conditions, undergoing opening of the benzoxazinone ring. 2-Bromo-2*H*-1,4-benzoxazin-3(4*H*)-ones show similar degradation under alkaline conditions, while replacement of Br at C(2) to give 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones was observed only under mild alkaline conditions. Mechanisms of ring opening and degradation to 2-aminophenol derivatives are proposed.

Tetrahedron lett 2008; 49: 3943-3945.

Simple and effective preparation of amino sulfonylureas from amino acids: application to the synthesis of amino sulfonylurea-containing peptidomimetics

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Several amino sulfonylureas have been synthesized, starting from amino acids. The synthetic procedure is simple affording high yields of products under mildconditions. Furthermore, it is shown that these compounds can be incorporated into a peptide sequence.

Synlett 2009; 3: 437-440.

Simple synthesis of 3-acetamido-ß-resorcylic acids as potential FabF and FabH inhibitors without using protecting groups

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A simple two-step strategy for the synthesis of 3-acetamido-β-resorcylic acids as potential platensimycin analogues was developed. It avoids the use of protecting group chemistry and starts from 2-aminoresorcinol, which is first N-acylated and then subjected to a modified Kolbe-Schmitt carboxylation to yield the desired 3-acetamido-β-resorcylic acids.

Synlett 2008; 13: 2036-2040.

Straightforward and Efficient Synthesis of (4R,6S)-4-(tert-Butyldimethylsiloxy)-6-(hydroxymethyl)tetrahydropyran-2-one

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A novel synthetic approach to (4*R*,6*S*)-4-(*tert*-butyldimethylsiloxy)-6-(hydroxymethyl)tetrahydropyran-2-one, a key precursor of statin side chain, is described. A prime feature of the presented strategy is the transformation of (4*R*,6*S*)-4-(*tert*-butyldimethylsiloxy)-6-(iodomethyl)-tetrahydropyran-2-one to an acetate ester derivative and subsequent cleavage of an acetate protection by applying homogeneous tin catalysis. Iodolactone used in the study is accessible by a new route in five steps from (*S*)-ethyl 4-chloro-3-hydroxybutanoate. This method overcomes many of the drawbacks associated with previously reported approaches. It gives the title compound in 21% over seven steps, which is the highest attained overall yield yet. The disclosed approach was realized in convenient and economical manner suitable for industrial use.

Tetrahedron 2008; 64: 9093-9100.

Synthesis of 1-C-linked diphosphate analogues of UDP-N-Ac-glucosamine and UDP-N-Ac-muramic acid

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UDP-*N*-acetyl-glucosamine and UDP-*N*-acetyl-muramic acid are two important cytoplasmic precursors of bacterial peptidoglycan. The convergent synthesis of their analogues is reported. The α-1-*C*-linked-*N*-acetyl-glucosamine was synthesized using microwave-assisted Keck radical allylation. Oxidation of alkene derivatives to the corresponding carboxylic acids allowed attacheminet to *O*- and *N*-sulfamoyluridine giving the stable diphosphate mimetics.

Tetrahedron lett.. 2007; 48: 1465-1468.

Synthesis of 3,5-disubstituted 1,2,4-oxadiazoles as peptidomimetic building blocks

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Twelve new 1,2,4-oxadiazole based compounds have been synthesized, using a simple and efficient synthetic route to afford enantiopure compounds in good yields.

Heterocycles 2008; 75: 1355-1370.

Synthesis of 8-hydroxyimidazo[1,2-a]pyridine-2-carboxylic acid and its derivatives

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Two new imidazo[1,2-*a*]pyridines, 8-hydroxyimidazo[1,2-*a*]pyridine-2-carboxylic acid (**4**) and ethyl 8-hydroxyimidazo[1,2-*a*]pyridine-2-carboxylate (**6**) were prepared *via* cyclization of 2-aminopyridin-3-ol (**1**) with bromopyruvic acid (**2**) and ethyl bromopyruvate (**3**), respectively. 8-Hydroxyimidazo[1,2-*a*]- pyridine-2-carboxylic acid (**4**) was successfully coupled with various amino acid derivatives *via* its active ester intermediate into the corresponding amides **22-27**. *O*-protected ethyl 8-hydroxyimidazo[1,2-*a*]pyridine-2-carboxylate **11** was transformed into its hydrazide **13**, acyl azide **14**, and amide **15** derivatives.

Tetrahedron 2009; 65: 659-665.

Synthesis of novel amphiphilic spin probes with the paramagnetic doxyl group in the polar region

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The use of ESR and specially designed spin probes has led to major breakthroughts in understanding the complexity of biological membranes. Research has been focused mainly on molecular events within the lipid bilayer, and few probes have been designed for studing events in the extracellular space near the membrane surface. We have prepared a series of amphiphilic spin probes in which an ethylene glycol type hydrophylic spacer was introduced between a hydrophobic anchor and the doxyl group, placing the latter above the membrane in the extracellular space. Furthermore, 2pΠ orbital, containing the unpaired electron of the nitroxide grouop, would be oriented perpendicular to the membrane surface, making it more useful for ESR investigations of structural and dynamics properties close to the membrane surface in different situations of the cell life.

Syntett 2008; 1155-1158.

Synthesis of Novel Bicyclic Nitroxides Using Partial Favorskii Rearragement

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3-Bromo-2,2,6,6-tetramethyl-4-oxopiperidine-1-oxyl was reacted with several *C*-nucleophiles to give novel bicyclic pyrrolidine nitroxides through Favorskii rearragement. Further reduction with sodium borohydride gave spin probes with free hydroxyl groups and under hursh reduction conditions allowed the Favorskii rearragement to proceed to completion.

Tetrahedron 2009; 65: 344-350.

Three-component one-pot synthetic route to 2-amino-5-alkylidenethiazol-4-ones

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A fast and straightforward three-component reaction to 2-amino-5-alkylidene-thiazol-4-ones is described. The one-pot methodology, reported for the first time, involves Knoevenagel condensation of aromatic aldehydes and rhodanine followed by displacement of the thiocarbonyl sulfur with primary or secondary amines in the same reaction mixture. The reactions were performed using a dedicated microwave reactor, which enabled short reaction times and easy work-up.

Tetrahedron: Asymmetry 2008; 19: 2265-2271.

Total synthesis of uridine diphosphat-N-actylmuramoyl-L-alanine

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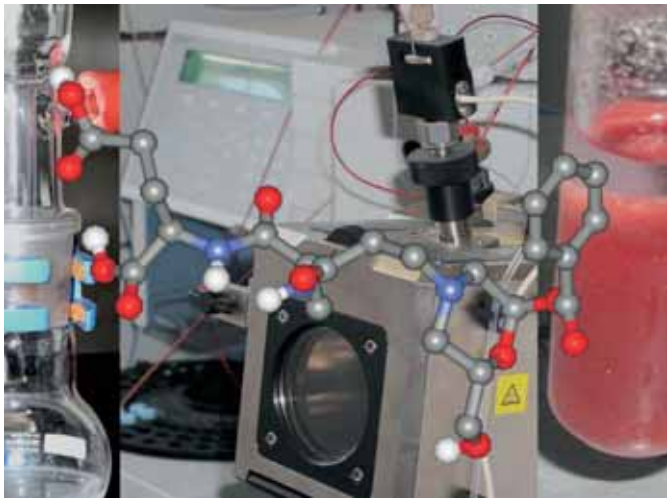
The firsrt total synthesis of uridine diphosphate *N*-acetylmuramoyl-L-alanine in 13% averall yield is presented. The 11-step synthetic route is based on the syntetic strategy used for the synthesis of uridine diphosphate *N*-acetylmuramic acid, the MurC ligase substrate. However, un unexpected amide bond cleavage under basic conditions demanded crucial modifications of the final synthetic step. The total chemical synthesis of MurD ligase substrate provides an excellent alternative to chemoenzymatic synthesis.

Science and art of chemical synthesis



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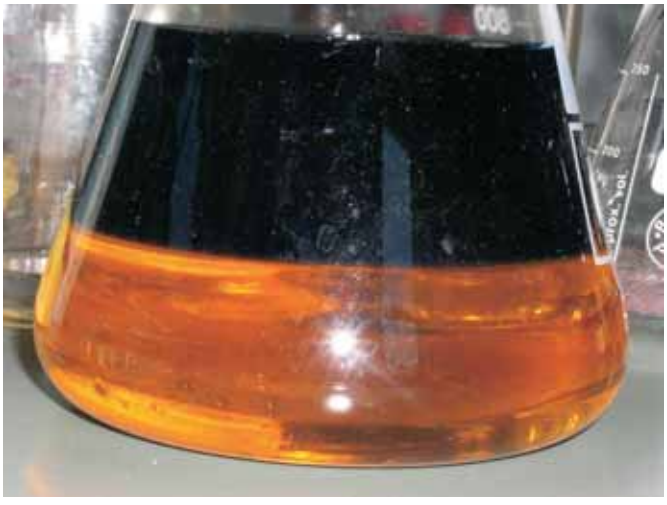

Science and art of chemical synthesis

Pharmacol Rep 2008; 60: 742-749.

Acute doxorubicin nephrotoxicity in rats with malignant neoplasm can be successfully treated with fullerenol C60(OH)24 via suppression of oxidative stress

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Oxidative stress has an important role in the pathogenesis of doxorubicin (DOX)-induced nephrotoxicity. The aim of this study was to investigate the nephroprotective effects of fullerenol (FLR) ($C_{60}(OH)_{24}$), an antioxidant agent, on DOX-induced nephrotoxicity.
The investigation was carried out on adult female Sprague Dawley outbred rats with chemically induced breast cancer (1-methyl-1-nitrosourea; 50 mg/kg; ip). Rats were divided into the following groups: control healthy, control cancer, DOX alone (8 mg/kg, ip, cancer), DOX plus FLR as a pre-treatment (8 mg/kg and 100 mg/kg, respectively, ip, cancer), and FLR alone (100 mg/kg, ip, cancer). At the end of the 2nd day after drug administration, blood and kidney tissues were taken for analysis. The activity of lactate dehydrogenase and α-hydroxybutyrate dehydrogenase as serum enzymes, as well as level of malondialdehyde, glutathione, glutathione peroxidase, glutathione reductases, catalase and superoxide dismutase, were determined.
DOX caused nephrotoxicity, but FLR pre-treatment prevented oxidative stress, lipid peroxidation and the disbalance of GSH/GSSG levels in kidney tissue caused by DOX. Our results confirm satisfactory nephroprotective efficacy of FLR in the acute phase of toxicity and encourage further studies regarding its use as a potential nephroprotector.

Technol Cancer Res Treat 2008; 7: 15-26.

Cardioprotective effects of fullerenol C60(OH)24 on a single dose doxorubicin-induced cardiotoxicity in rats with malignant neoplasm

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The therapeutic utility of the anthracycline antibiotic doxorubicin is limited due to its cardiotoxicity. Our aim was to investigate the efficacy of fullerenol $C_{60}(OH)_{24}$ in preventing single, high-dose doxorubicin-induced cardiotoxicity in rats with malignant neoplasm. Experiment was performed on adult female Sprague Dawley rats with chemically induced mammary carcinomas. The animals were sacrificed 2 days after the application of doxorubicin and/or fullerenol, and the serum activities of CK, LDH and α-HBDH, as well as the levels of MDA, GSH, GSSG, GSH-Px, SOD, CAT, GR and TAS in the heart, were determined. The results obtained from the enzymatic activity in the serum show that the administration of a single dose of 8 mg/kg in all treated groups induces statistically significant damage. There are significant changes in the enzymes of LDH and CK ($p < 0.05$), after an i.p. administration of doxorubicin/fullerenol and fullerenol. Comparing all groups with untreated control group, point to the conclusion that in the case of a lower α-HBDH/LDH ratio, results in more serious the liver parenchymal damage. The results revealed that doxorubicin induced oxidative damage and that the fullerenol antioxidative influence caused significant changes in MDA, GSH, GSSG, GSH-Px, SOD, CAT, GR and TAS level in the heart ($p < 0.05$). Therefore, it is suggested that fullerenol might be a potential cardioprotector in doxorubicin-treated individuals.

Biomaterials 2008; 29: 3451-3460.

Potential Hepatoprotective Effects of Fullerenol C60(OH)24 in Doxorubicin-induced Hepatotoxicity in Rats With Mammary Carcinomas

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The aim of this study was to investigate the potential protective role of fullerenol $C_{60}(OH)_{24}$ on doxorubicin-induced liver toxicity using in vivo (female Sprague-Dawley rats) and in vitro (human hepatocellular carcinoma - HepG2; colorectal adenocarcinoma cell lines - Caco-2) approaches. The first (healthy control) and second (control with chemically induced mammary carcinomas) group received saline only. The third, fourth and fifth group (all with breast cancer) were injected (i.p.) with a single dose of doxorubicin (8 mg/kg), doxorubicin/fullerenol (100 mg/kg of fullerenol 30 min before administration of 8 mg/kg doxorubicin) and fullerenol (100 mg/kg), respectively. Two days after treatment, the rats were sacrificed. Results showed that treatment with doxorubicin alone caused significant changes in the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alpha-hydroxybutyrate dehydrogenase (α-HBDH), as well as in the levels of malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GSH-Px), total antioxidant status (TAS), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) in the liver tissue. These effects were significantly reduced for all investigated parameters by pre-treatment with fullerenol but not for the MDA and GSH level. The HepG2 and Caco-2 cell lines were continuously treated with fullerenol for 12, 24, 48 and 96 h at concentrations of 10 and 44 g/mL. With the aim of evaluating the modulating activity of fullerenol on doxorubicin induced hepatotoxicity, the cell lines were simultaneously treated with doxorubicin (1 μM; 5 μM) and fullerenol (10 μg/mL; 44 g/mL) in different combinations. When the cells are treated with 5 μM doxorubicin along with the fullerenol, we can see a significant improvement of the cell capability during the entire time-line. We can conclude that fullerenol has cytotoxic effects on HepG2 by itself, but when the oxidative stress is too high the cytotoxic effects of fullerenol are overcome by its protective role as a strong antioxidant compound.

Biomaterials 2009; 30: 1184-1196.

Protective Effects of Fullerenol C60(OH)24 against Doxorubicin-induced Cardiotoxicity and Hepatotoxicity in Rats with Colorectal Cancer

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The effects of fullerenol $C_{60}(OH)_{24}$ (Frl) at doses of 25, 50, and 100 mg/kg/week (for a time-span of three weeks) on heart and liver tissue after doxorubicin (Dox)-induced toxicity in rats with colorectal cancer were investigated. In the present study, we used an in vivo Wistar male rat model to explore whether Frl could protect against Dox-induced (1.5 mg/kg/week for three weeks) chronic cardio- and hepato- toxicity and compared the effect with a well-known antioxidant, vitamin C (100 mg/kg/week for three weeks). According to macroscopic, microscopic, hematological, biochemical, physiological, pharmacological, and pharmacokinetic results, we confirmed that, at all examined doses, Frl exhibits a protective influence on the heart and liver tissue against chronic toxicity induced by Dox.

Technol Cancer Res Treat 2008; 7: 497-516.

Recent advances in protection against doxorubicin-induced toxicity

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Anthracycline antibiotics are among the most effective and commonly used anticancer drugs. Unfortunately, their clinical use is restricted by dose-dependent toxicity. Doxorubicin is an anthracycline antibiotic and cytotoxic (antineoplastic) agent. It is commonly used against ovarian, breast, lung, uterine and cervical cancers, Hodgkin's disease, soft tissue and primary bone sarcomas, as well against in several other cancer types. It has been shown that free radicals are involved in doxorubicin-induced toxicity. Doxorubicin causes the generation of free radicals and the induction of oxidative stress, associated with cellular injury. This review illustrates recent applications of different natural products, drugs, drug delivery systems and approaches for protection against doxorubicin-induced toxicity (2006-present).

Toxicol Mech Methods 2009; 19: 24-28.

Activity of Antioxidative Enzymes in Erythrocytes after Single Dose Administration of Doxorubicin in Rats Pretreated by Fullerenol C60(OH)24

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Earlier investigation of fullerenol, $C_{60}(OH)_{24}$, features, in vitro, showed that fullerenol have a strong antioxidative potential. In this work, we examined the influence of fullerenol as a potential antioxidative protector on doxorubicin induced oxidative stress in blood of rats through investigation of the activity of glutathione dependent enzymes (glutathion-S-transferase and glutathion peroxidase). Influence of fullerenol on blood cells number (leukocytes and erythrocytes) as well as haemoglobin content after single dose administration of doxorubicin was also observed. Experiments were performed on adult mail Wistar rats, distributed in six groups, each containing eight individuals. Doxorubicin was administrated i.v. (tail vein) in single dose of 10 mg/kg. Fullerenol $C_{60}(OH)_{24}$ in treated animals was administrated i.p. (in doses 50, 100, 200 mg/kg) 30 min. before application of doxorubicin. Control group (intact animals) were given saline (1 ml/kg). One group of animals was treated only with fullerenol (100 mg/kg i.p.). Each experiment was repeated twice and animals were sacrificed after 2 and 14 days after the treatment. Our results may indicate that fullerenol induces the decrease in antioxidative capacity of erythrocytes in oxidative stress conditions, whereas without doxorubicin, the application of fullerenol did not induce any changes in enzyme activity of erythrocytes. Results of GST activity might indicate that the 50 mg/kg are not sufficient to protect from doxorubicin toxicity, while 200 mg/kg might be toxic for animals, according to the increase in GST activity.

Int J Pharm 2008; 363: 183-191.

Effect of resveratrol incorporated in liposomes on proliferation and UV-B protection of cells

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The possibility of improving the efficacy of resveratrol, a polyphenol with strong antioxidant and freeradical scavenging properties, on cell proliferation and photoprotection by liposomal incorporation was investigated. Oligolamellar vesicles of different lipid compositions, loaded with resveratrol, were prepared and characterized by evaluating size, zeta potential, incorporation efficiency, electron microscopy and stability over 60 days. The effect of free and liposomal resveratrol on the viability of HEK 293 cells and their photoprotection after UV-B irradiation was assessed by the MTS method. Resveratrol decreased the cell viability at 100 μM concentration, while at 10 μM increased cell proliferation and also achieved the most effective photoprotection. Photomicrographs of the treated cells from inverted light and fluorescence microscopy demonstrated resveratrol effectiveness at 10 μM, as well as its toxicity at higher concentrations, based on changes in cell shape, detachment and apoptotic features. Interestingly, liposomes prevented the cytotoxicity of resveratrol at high concentrations, even at 100 μM, avoiding its immediate and massive intracellular distribution, and increased the ability of resveratrol to stimulate the proliferation of the cells and their ability to survive under stress conditions caused by UV-B light.

ChemBioChem 2008; 9: 1921-1930.

Synthesis of novel DC-SIGN ligands with an [alpha]-fucosylamide anchor

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The dendritic cell-specific intercellular adhesion molecule (ICAM) 3-grabbing nonintegrin (DC-SIGN) is a C-type lectin that appears to perform several different functions. Besides mediating adhesion between dendritic cells and T lymphocytes, DC-SIGN recognizes several pathogens some of which, including HIV, appear to exploit it to invade host organisms. The intriguing diversity of the roles attributed to DC-SIGN and their therapeutic implications have stimulated the search for new ligands that could be used as biological probes and possibly as lead compounds for drug development. The natural ligands of DC-SIGN consist of mannose oligosaccharides or fucose-containing Lewis-type determinants. Using the known 3D structure of the Lewis-x trisaccharide, we have identified some monovalent alpha-fucosylamides that bind to DC-SIGN with inhibitory constants 0.4-0.5 mM, as determined by SPR, and have characterized their interaction with the protein by STD NMR spectroscopy. This work establishes for the first time alpha-fucosylamides as functional mimics of chemically and enzymatically unstable alpha-fucosides and describes interesting candidates for the preparation of multivalent systems able to block the receptor DC-SIGN with high affinity and with potential biomedical applications.

Bioorg Med Chem 2008, 16, 3926-3932.

Design and synthesis of N-(3, 5-difluoro-4-hydroxyphenyl)benzene-sulfonamides as aldose reductase inhibitors

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N-(3,5-Difluoro-4-hydroxyphenyl)benzenesulfonamide (4) and its derivatives 5-7 were prepared as putative bioisosteres of the previously reported aldose reductase inhibitors, which are the N-benzenesulfonylglycine derivatives I-IV. The in vitro aldose reductase inhibitory activity of the prepared compounds is higher than that of the respective glycine derivatives. Furthermore, the parent compound 4 reveals high antioxidant potential. Additionally, the intestine permeability of 4 is determined, and there is initial evidence that there is an operating influx mechanism. Overall, the data indicate that the presented chemotype could serve as a core structure forthe design of putative pharmacotherapeutic agents, aiming to the long-term complications of diabetes mellitus.

Naturwissenschaften 2007; 94: 935-939.

Physicochemical and physiological basis of dichromatic colour

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Out of three perceptual characteristics of the colour of any substance, the hue depends mostly on the spectral properties of a substance, while the brightness and saturation depend also on the concentration of a substance and its thickness. Here, we report that evident change of the hue of the colour (i.e., from green to red) is due to a change in concentration or the thickness of a layer in some exceptional substances such as pumpkin seed oil or an aqueous solution of bromophenol blue. In some regions of Central Europe, salad dressing is made preferably with the pumpkin seed oil, which has a strong characteristic nut-like taste and remarkable properties of the colour: it appears red in a bottle, but green when served as a salad dressing. The colour of the pumpkin seed oil was previously described as brownish yellow, dark green, dark green to red ochre or dark reddish brown to light yellow green. We elucidated the physicochemical and physiological basis of such dichromatism by Beer-Lambert law and by the characteristics of human colour perception. Our concept was corroborated by the outcome of calculations of colour from spectral properties using colour matching functions. We found that dichromatism is observed if the absorption spectrum of any substance has at least two local minima: one wide but shallow and one narrow but deep local minimum.

Chem Phys Lipids 2008; 156: 17-25.

An ESR characterization of micelles and vesicles formed in aqueous dacanoic acid/sodium decanoate systems using different spin labels

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Aqueous decanoic acid/sodium decanoate systems were studied as a function of pH and concentration up to 0.3 M decanoic acid/sodium decaoate, by electron spin resonance (ESR) spectroscopy using three different amphiphilic spin labels. The distribution of spin labels between vesicles and micelles as well as their dynamics properties were determinated by quantitative analysis of the ESR spectra using two novel simulation software packages. Rotational correlation time of the labels in micelles wasfound to increase with increasing pH, with substantial increase in the region where vesicles were formed (7.8<pH<8). In the interval 6.5<pH<7.8, the coexistence of vesicles and micelles was observed. Presence of vesicles was confirmed by captured aqueuos volume, determined independently with a hydrophilic spin label. The ESR measurement indicate that decanoic acid vesicles formation observed as the concentration is increased between 0.01 M and 0.03 M at pH 7.0 most likely occurs *via* the formation of micelles which remain in coexistence with the vesicles, even if the concentration is well above these values.

Drug Dev Ind Pharm 2009; 35: 114-117.

Acido-Basic Properties of Proton Pump Inhibitors in Aqueous Solutions

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The pharmacological characteristics of proton pump inhibitors are related to theirprotolytic behavior estimated by their pK_a values. Lansoprazole is a potent anti-acid drug from this group. Because of its poor stability a rapid spectrophotometric method was developed for the determination of its pK_a values. Three pK_a values were obtained: an acidic pK_{a1} = 8.84 and two basic, pK_{a2} = 4.15 and pK_{a3} = 1.33. These pK_a values were discussed from the point of lansoprazole structure and instability with the aim of locating basic and acidic moieties in the molecule of proton pump inhibitors. They were also compared with experimentally determined pK_a values from the literature and with some pK_a values calculated by different programs.

J Agric Food Chem 2008; 56: 9114–9120.

Selenium Species in Selenium-Enriched and Drought-Exposed Potatoes

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The aim of this work was to study selenium (Se) speciation in the potato (Solanum tuberosum L.) cultivar Desiree, enriched in Se by foliar spraying with a water solution containing 10 mg of Se/L in the form of sodium selenate. Four combinations of treatments were used: well-watered plants with and without Se foliar spraying and drought-exposed plants with and without Se foliar spraying. Water-soluble Se compounds were extracted from potato tubers by water or enzymatic hydrolysis with the enzyme protease XIV, amylase, or a combination of protease XIV and amylase. Extraction was performed using incubation at a constant temperature and stirring (37 °C at 200 rpm) or by ultrasound-assisted extraction (300 W), using different extraction times. Separation of soluble Se species (SeCys2, SeMet, SeMeSeCys, selenite, and selenate) was achieved by ion-exchange chromatography, and detection was performed by inductively coupled plasma-mass spectrometry (ICP-MS). Results showed that the concentration of selenate extracted was independent of the enzymatic extraction technique (approximately 98 ng/g for drought-exposed and 308 ng/g for well-watered potato tubers), whereas the extraction yield of SeMet changed with the protocol used (10-36%). Selenate and SeMet were the main soluble Se species (representing 51-68% of total Se) in potato tubers, regardless of the growth conditions.

Int J Clin Pract 2007; 61: 1979–1988.

Cost-effectiveness of antipsychotics for outpatients with chronic schizophrenia

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Aims: The aim of the present analysis was to evaluate the cost-effectiveness of alternative treatments for outpatients with chronic schizophrenia from the healthcare payer's perspective.
Methods: Decision analysis was used to evaluate the costeffectiveness of the following antipsychotic drugs: amisulpride, aripiprazole, haloperidol (oral formulation), haloperidol (depot formulation), olanzapine, quetiapine, risperidone (oral formulation), risperidone (depot formulation) and ziprazidone. Clinical and economic outcomes were modelled over 1-year time horizon. Effectiveness was measured as a percentage of patients in remission. Clinical parameters used in the model included compliance rates, rehospitalisation rates for compliant and non-compliant patients, duration and frequency of hospitalisation, and adverse event rates. One-way sensitivity analysis was performed to test the robustness of the model.
Results: The most effective treatment was treatment with olanzapine where 64.1% of patients remained in remission. The least effective treatment was treatment with quetiapine where 32.7% of patients remained in remission. Overall costs ranged from 3726.78 for haloperidol to 8157.03 for risperidone in depot formulation. Inpatient costs represented the major part of costs for most of antipsychotic drugs. Typical antipsychotic drugs had substantially smaller outpatient costs (6.5%) compared with atypical antipsychotics (37.9%). In the base case scenario the non-dominated treatment strategies were haloperidol, haloperidol decanoate and olanzapine. Additionally, risperidone can also be considered to be part of the efficient frontier based on the sensitivity analysis results.
Conclusion: Among second-generation antipsychotics, which have a better safety profile than first-generation antipsychotics, olanzapine and risperidone showed to be the most cost-effective treatment strategies for outpatient treatment of chronic schizophrenia.

Int J Public Health 2009; 54: 35–39.

Body shape, body size and cigarette smoking relationships

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Objectives: The aim of the present study was to explore whether smoking is positively related with the abdominal obesity.
Methods: Cross-sectional data was analyzed from a random sample of 1,342 subjects, stratified by their age and gender.
Cigarette smoking habits were assessed and anthropometric measures obtained during the health examination.
Results: Statistically significant differences in waist circumference and waist-hip ratio were found among males in some of the age groups, body mass index however, was lower in older smokers. There were no differences between female smokers and non-smokers in all parameters.
Conclusion: The results of the study do not support the hypothesis that smoking affects an abnormal fat distribution profile predominantly in the form of central adiposity as reported earlier.

Bioorg Med Chem Lett 2007; 17: 6330-6334.

New biologically active epidioxysterols from Stereum hirsutum

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From the fungus *Stereum hirsutum* have been isolated and identified two new epidioxysterols 1, 4, together with two known ones 2 and 3. Their structures were elucidated on the basis of spectroscopic analysis and chemical reactions. Epidioxysterols 1–4 have been shown to possess a significant activity against *Mycobacterium tuberculosis.*

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