CECE 2008

5th international interdisciplinary meeting on bioanalysis

November 24 – 25

Hotel Continental, Brno, Czech Republic

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Organized by:
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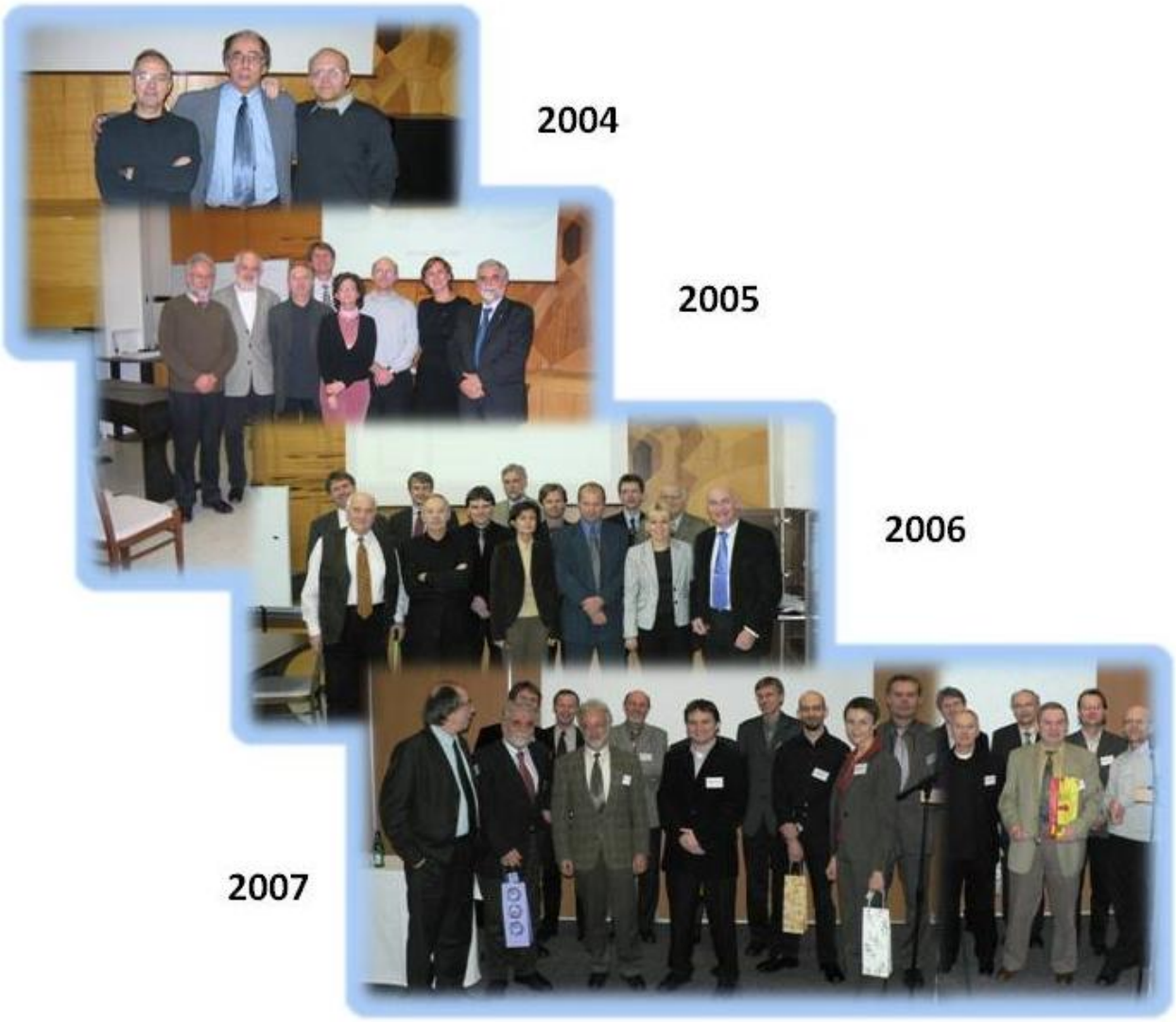
Introduction

Welcome to CECE 2008. Since 2004, when a one day seminar was given by Dusan Kaniansky, Ernst Kenndler and Bob Gas in the conference room of the Institute of Analytical Chemistry, we are now in the 5th year of CECE. Today, the meeting has a standard conference format with invited lectures and poster sessions. Yet, we want to stay unique. Our goal is bringing together scientists who may not meet at specialized meetings, promote informal communication of researchers from different disciplines and map the current status of the fields shaping the bioanalytical science. The organizers want to thank you for your participation and hope that you will enjoy the scientific presentations as well as personal contacts and informal discussions.



Program

Monday November 24, 2008

9:00 – 9:15 **Conference opening**

9:15 – 9:45 **Daniel R. Knapp**
Microfluidic system for ESI (DESI) and MALDI (LDI) shotgun proteomic analysis

9:45 - 10:15 **Holger Becker**
CE with contactless conductivity detection in polymer chips

10:15 – 10:45 **Jörg P. Kutter**
Combining microfluidics with small angle X-ray scattering for structural analysis of proteins: small chip – large detector – perfect marriage!

10:45 – 11:15 **Pavel Neužil**
Development of a universal Lab-on-a-Chip system

11:15 – 11:45 **Alejandro Cifuentes**
New metabolomics approaches for transgenic food analysis based on capillary electrophoresis-mass spectrometry

11:45 – 13:45 **Lunch break – poster session**

13:45 – 14:15 **Vladimír Havlíček**
Ambient mass spectrometry on FTICR

14:15 – 14:45 **Jiří Homola**
Surface plasmon resonance sensors and their bioanalytical applications

14:45 – 15:15 **Sven Preuss**
3D microstructuring using maskless laser lithography

15:15 - 15:45 **Staffan Nilsson**
Nanoparticle-based CEC and airborne chemistry

19:00 Conference dinner - hotel Continental

Tuesday November 25, 2008

9:00 – 9:30 Dušan Kaniansky
Some specific features of chip electrophoresis with column-coupling in ultra(trace) analysis

9:30 – 10:00 Jana Křenková
Monolithic platform for analysis of therapeutic antibodies

10:00 – 10:30 Mirek Macka
Shedding LED light on synergies between analytical science, miniaturisation, photochemistry, and photonics

10:30 – 11:00 Ewa Klodzinska
Electromigration and molecular biology methods in determination and identification of clinical strains of pathogenic bacteria

11:00 – 11:30 Wolfgang Lindner
HILIC type selectivity in LC – HYPE or HOPE

11:30 - 12:00 Ferenc Kilár
Fast and sensitive detection methods for endotoxin analysis in microchips

12:00 – 14:00 Lunch break – poster session

14:00 – 14:30 Andras Guttman
Sample preparation issues in complex carbohydrate analysis by capillary gel electrophoresis

14:30 – 15:00 Martin Polcik
Process analytical technology – new opportunities for pharma and related industries

15:00 – 15:30 Bohdan Růžička
Project of the Central European Synchrotron Laboratory

15:30 Conference closing

Abstracts

Lectures

Microfluidic system for ESI (DESI) and MALDI (LDI) shotgun proteomic analysis

D.R. Knapp , J. Liu, R. Nayak, A.K. Sen, G. Chen and D.J. Higbee
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Shotgun proteomic analyses using liquid chromatography (LC) electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS) have been shown to observe complementary sets of peptides with incomplete overlap. For maximal proteomic coverage, it is desirable to employ both modes of analysis. We are developing a polymer-based microfluidic LC device designed to interface to both ESI and MALDI MS as well as to a new dual mode ESI - DESI MS platform. These developments combined promise to increase the depth of coverage in proteomic analysis using shotgun methods.

Nickel masters for microfluidic devices were prepared by double layer microlithography on nickel wafers using SU-8 photoresist and nickel plating. Microfluidic devices were prepared by hot embossing in cyclic olefin copolymer wafers and heat bonding cover plates. Nanoporous alumina thin films were prepared by vacuum evaporating aluminum onto glass slides and anodizing.[1] A thin film of gold was applied to the alumina by sputtering. MALDI and LDI spectra were obtained on an Bruker Autoflex III instrument. ESI and DESI spectra were obtained on a Thermo LTQ ion trap instrument using Thermo Nanospray ESI and in - house constructed DESI sources.[2] The first dimension ionization - based separation (strong cation exchange or isoelectric focusing) is performed offline, and the fractions collected onto a microfluidic device consisting of an array of monolithic reversed phase LC columns. The columns are eluted simultaneously in the second dimension separation, and the separation fractions collected in parallel onto a nanoporous alumina sample plate using electrostatic transfer.

As an example of a peptide mixture analysis, a BSA tryptic digest gave the following results (numbers are numbers of fragment peptides observed):[3]

The LDI/ DESI combination gives peptide coverage comparable to MALDI/ESI, but with the added advantage of a common sample format, thereby enabling deeper analysis of a sample spot that would otherwise be only amenable to MALDI analysis.

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CE with contactless conductivity detection in polymer chips

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While chip-based methods of capillary electrophoresis have allowed for the miniaturization of the separation column, subsequent elements of the analytical system, namely the detection unit, usually remain bulky and complicated. This is especially true in the case of the most frequently used detection method of laser-induced fluorescence (LIF). In order to obtain a truly portable system, we have developed a chip-based CE system which utilizes capacitively coupled contactless conductivity detection (C^4D) [1,2] as detection method, operating at 4 MHz which is significantly higher than in previously reported C^4D systems. This allows the complete CE unit, including all the electronics (high-voltage power supplies, detection electronics) and the chip itself to fit into a metal box with dimensions 19 cm × 12 cm × 8 cm [3]. Main analytical target is the identification of ionic species in foodstuffs like water, milk or wine.

In order to allow for a disposable chip, the chips are injection molded from polymethyl-methacrylate (PMMA). The fluidic reservoirs with a fluid volume of 70 µl have already been integrated into the chip to avoid chip additional assembly steps [4]. The detection is realized by C^4D with the electrodes on the outside of the chip in order to avoid sample contact with the detection electrodes. The electrodes are fabricated by metal vapor deposition, alternatively with screen-printing. The separation channel has the dimensions 50 µm width, 50 µm in height with a separation length of 70 mm; the electrodes have a width of 200 µm with an electrode gap for measuring of 250 µm.

We have been able to separate relevant species of ions, organic acids and sugars in mineral water, wines and milk. Typical separation voltages are between 3 and 4 kV, with typical ionic concentrations <100µM in water and acid concentrations in wine of < 0.5 mM. The separations could be carried out typically in 60-120 sec. The high operating frequency reduces the noise to <10 µV.

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Acknowledgements.
We would like to thank Dr. W. Hoffmann and Dr. H. Mühlberger from Forschungszentrum Karlsruhe for the preceeding development and Thomas Clemens from Clemens GmbH, Waldbüttelbronn for the system assembly.

Combining microfluidics with small angle X-ray scattering for structural analysis of proteins: small chip – large detector – perfect marriage!

Kutter, J. P. [1]; Snakenborg, D. [1]; Nielsen, S. S. [1, 2], Toft, K. N. [1, 2]; Jeppesen, M. G. [2], Jacobsen, J. K. [3], Arleth, L. [2] and Vestergaard, B. [2]
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Proteins are dynamic macromolecules that can undergo conformational changes or form complexes with other biological molecules. Elucidation of these suprastructural features lies at the heart of an understanding of the structure:function relationship. Still, such features are difficult to investigate using established methods for structural characterization. One reason is that in order to get a comprehensive picture it is necessary to examine the macromolecules under many different solution conditions, which today is very demanding both in terms of time and sample consumption. Basically, there are an infinite number of possibly relevant experiments, and a structural biologist essentially navigates blindfolded in this multidimensional experimental space.

Here, we describe the development of a system combining microfluidics for high-throughput screening of a large solution parameter space while using minute amounts of rare biomacromolecules, and small-angle X-ray scattering (SAXS) as the tool for elucidating suprastructural features. Such a system also has the potential to enable screening of, e.g., formulation of bio-drugs and solubilization of membrane proteins. Furthermore, powerful software for automated data analysis is developed, allowing direct feedback to the microfluidic front-end. Thus, rational navigation in the multidimensional experimental space in an iterative way is possible allowing pinpointing of structurally and functionally relevant areas of investigation, borders between various functional states, or structurally stable experimental windows of potentially labile biological systems. Ultimately, the SAXS/μTAS system enables time-resolved studies of biological processes, using already established microfluidic techniques in combination with our high-throughput screening.

A polymer-based prototype microfluidic system has been developed featuring simple mixing capabilities for protein and buffer solutions and an integrated X-ray transparent analysis chamber with only 200 nL probe volume at an interaction pathlength of 1 mm. First successful protein measurements using such small volumes have been performed on a synchrotron beamline. The versatility of the system has been tested by running automated series of protein dilution and protein unfolding experiments[1]. Conditions were accurately prepared by the microfluidic front-end, while the results were monitored using the SAXS technique and an automated, optimized data treatment. A second-generation device is currently being developed, featuring a higher degree of integration and added functionalities.

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Development of a universal Lab-on-a-Chip system

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In this talk, I will present an overview of our research activities to develop a simple and universal LOC system. Originally, the aim of our work was to develop a cheap and portable real-time reverse transcription polymerase chain reaction (RT-PCR) system to tackle potential outbreaks of avian flu or similar diseases. In sequence, we have demonstrated one of the world's cheapest PCR systems, capable of performing 40 PCR cycles in less than 6 minutes and thus one of the fastest systems as well. Finally, we have integrated the PCR with a fluorescence detection unit. Its performance was demonstrated by detecting of RNA of H5N1 virus as well as the SARS virus using unprecedented 8 minutes real-time RT-PCR. During the PCR development we have come up with a new method for quantitative multiplexing of a PCR (RT-PCR) using SYBR-Green as well as a system for an ultrafast sample heating suitable to break spore shells to release their DNA.

Most of the LOC systems require detecting small currents originating either from photodetectors or other devices (e.g. nanowires) and being able to control temperature is always a bonus for any bio application. It was only natural to expand the original real-time RT-PCR device into a universal modular LOC system. We have added a high voltage controller to perform capillary electrophoresis (CE) and a stepper motor controller to perform PCR in space domain, possibly able perform fluid pumping. I will show a few "clones" of the universal LOC systems, such as localized surface plasmon resonance (LSPR), real-time RT-PCR stationary sample,real-time RT-PCR with movable sample, high voltage source performance for the CE as well as results from electrochemical system.

New metabolomics approaches for transgenic food analysis based on capillary electrophoresis-mass spectrometry

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At present, one of the principal research lines in Food Science and Technology is the development of new functional foods. A food can be considered ‘‘functional’’ if, besides its nutritious effects, it can improve the state of health or well-being or reduce some disease risk. In our laboratory, we have studied for years different natural sources of functional ingredients including plants, spices, algae, etc. Nowadays, the use of genetically modified organisms (GMOs) has seen a great increase in agriculture and food industry. Thus, genetic engineering is mainly used to improve resistance of crops to plagues or pesticides. However, in the new generation of GMOs significant changes in other constituents will be deliberately introduced to generate new functional foods (e.g., increasing fatty acids, amino acids content, polyphenols, vitamins, or reducing undesirable constituents, etc), requiring the development of more powerful and informative analytical procedures. In this work, an original analytical strategy is proposed able to provide information on the composition of GMOs based on metabolomics studies carried out by capillary electrophoresis-mass spectrometry (CE-MS). The goal of this work is, therefore, to present the results from a comparative profiling study of metabolites found in transgenic varieties vs. their corresponding isogenic wild lines grown under identical conditions [1,2]. To do this, a complete analytical strategy is developed that combines metabolites extraction from samples, separation by capillary electrophoresis and chemical characterization by on-line electrospray-time of flight-mass spectrometry (CE-TOF-MS).

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Ambient mass spectrometry on FTICR

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We will report on the development of an ambient ion source, which we designed for Dual ion source on the FTICR instrument (Bruker Daltonics). This source can be operated in desorption electrospray (DESI), atmospheric chemical ionization (APCI), atmospheric pressure photoionization (APPI) and online microelectrospray (ESI) modes. In addition to sensitivity gain (15x compared to Combi-I ion source, *J Mass Spectrom.* 2008; 43: 196-203) our motivation was to utilize the mixing of internal reference calibrant ions generated by MALDI to get accurate ambient data. The source was implemented on a 9.4T FTMS system by constructing an electronically controlled source network comprising six linear moving stages and one rotating stage. A three-dimensional linear stage was used to accommodate samples, while another 3D linear stage equipped with rotating stage was used as a spray mount. In addition to changes in API part of mass spectrometer (two separated ion funnels) a new heated adapter was added resembling an ion vacuum cleaner. Also the base angle of the slide (glass, teflon, paper, TLC, etc.) with the deposited sample can be accurately adjusted now towards the sampling orifice of mass spectrometer. Original ESI nanosprayer (Agilent technologies, Palo Alto, USA) is used now instead of the previously used T-shape homemade sprayer. The current source is also aimed for mass imaging experiments. Less polar molecules not amenable to MALDI analysis (e.g. small lipids) could be analyzed in tissue sections. For reliable 2D imaging experiments robustness of the mechanical part represents an absolute prerequisite for successful DESI measurements. The current source is controlled by an independent console. The electronics responds to the duration of the movement commands: short commands elicit 5 micron steps; longer commands result in an exponential increase in the moving speed. PC-version controlled by LabView 8.5 software (National Instruments, USA) is in development. In this presentation we also will show new applications (e.g. the use of nanoparticles for desorption from surfaces, LC/MS attempts, etc.), performance evaluation data, as well as selected on-chip applications in APCI and APPI regimes.

Acknowledgement: Ministry of Education, Youth and Sports of the Czech Republic (LC07017) and IMIC institutional research concept (AVOZ50200510).

Surface plasmon resonance sensors and their bioanalytical applications

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Optical affinity biosensors are devices that incorporate a biological recognition element (*e.g.* antibody) which specifically recognizes a particular analyte and an optical transduction system which allows observation and quantification of the interaction between the analyte and the biomolecular recognition element. In the last decade we have witnessed development of numerous optical transduction methods, including both label-based methods such as fluorescence spectroscopy and label-free methods such as optical interferometry, spectroscopy of guided modes of optical waveguides, and surface plasmon resonance. Label-free optical biosensors exhibit numerous advantageous features. They allow direct and rapid measurements and enable sensitive and non-invasive determination of analytes in their natural environments.

This contribution reviews the present state of the art and recent advances in the development of surface plasmon resonance (SPR) sensors [1-2] and presents selected results of SPR sensor research at the Institute of Photonics and Electronics, Prague. The developments discussed in detail include mobile SPR sensors for field use, miniature fiber optic SPR probes for localized measurements, and SPR sensor platforms for parallelized observation of biomolecular interactions. Examples of applications of SPR sensors for detection of chemical and biological analytes related to medical diagnostics [3] (hormones, antibodies), environmental monitoring [4] (endocrine disrupting compounds), food safety and security [5] (pathogens and toxins) are also given.

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3D microstructuring using maskless laser lithography

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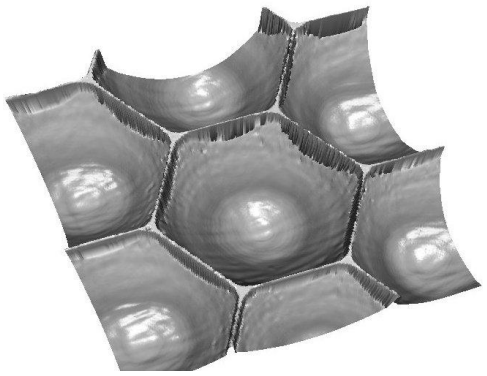
Direct Write Laser lithography (DWL) is an established technology for the creation of microstructures down to lateral dimensions of about 500nm. These systems use a focused laser beam to write structures that are defined by an electronic design directly onto a photoresist coated substrate. In addition to direct writing and the production of photomasks the maskless exposure technology can create arbitrary surface profiles in photoresists by modulation of the laser intensity [1]. The main applications for this 3D structuring are micro-optical components like micro lens arrays or blazed gratings, but also structures in the multi-level MEMS fabrication and the production of microfluidic devices.



A variety of structures have been created in different photoresists by maskless gray scale lithography. For each application the exposure and process parameters have to be optimized to achieve the desired surface profile in the photoresist. This becomes increasingly difficult in thicker resists above 50µm, which have to be coated in several layers and then have to be exposed multiple times. Small variations in the laser intensity distribution or in the development process parameters can lead to very different results. The gray scale exposures were done on standard positive photoresists but also on the negative photoresist SU8 that offers good chemical, mechanical and optical properties for different applications [2,3]. Because SU8 is a negative photoresist the exposures had to be made from the backside of the substrate, exposing through the glass.

Additional software and exposure strategies have been implemented into the laser lithography systems to alleviate the design process and increase the quality of the exposed structures. There are several methods to increase the surface quality at the cost of write speed. With full optimization a surface roughness below 10nm was achieved.

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Nanoparticle-based CEC and airborne chemistry

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The use of lipid-based liquid crystalline nanoparticles (~100 nm) as pseudostationary phase (PSP) in continuous full filling CEC/LIF for protein separation at neutral pH, without organic modifier and in a non-coated capillary (effective length 6.7 cm) will be described [1]. Equally charged, single amino acid substituted green fluorescent protein (GFP) mutants were separated utilizing high tricine concentrations to promote hydrophobic interactions. One trend in chromatography has been towards smaller stationary phase particles [2-3], *i.e.* submicron particles (*e.g.* UPLC). Traditionally, CEC has been performed using packed, open-tubular or monolithic columns. To minimize carry-over effects and column regeneration CEC can be performed with nanoparticles suspended in the electrolyte [2-3]. In addition, retaining frits and complicated packing procedures are avoided. Nanoparticles possess a favourable surface-to-volume ratio, which can allow for high chromatography efficiencies. Dextran-coated nanoparticles have been used in continuous full filling CEC/ESI-MS achieving highly efficient separations of small, neutral analytes, *i.e.* dialkylphthalates (up to 700 000 plates /m for retained analyte) [4]. An orthogonal electrospray interface was used to prevent the nanoparticles from entering the mass spectrometer.

Techniques for chemical analysis based on the use of levitated drops, suitable for the study of intra and extracellular reactions at single or few cell levels will be described. New insights in biomedicine and related areas require the parallel development of new analytical methods. The airborne system combined with MS was used to acquire data on single Langerhans islet and further on β-cell metabolism, at the few cells level, associated with stimulation using acetylcholine and increasing extracellular glucose concentration by droplet evaporation. The results obtained are scrutinized with known metabolism of islets and β-cells. In response to both acetylcholine and elevated glucose concentration, rapid insulin release was observed, together with other compounds, such as c-peptide and amylin. The low attomole LOD demonstrates the potential of the described method. We are currently developing experiments that allow us to compare the response of the adipocytes to the insulin produced by normal individuals and the glycated insulin produced by diabetic ones. Cell-containing 100-500 nL drops are levitated in an ultrasonic field. Cells and reagents are added to the drop using flow-through dispensers, and the cell reactions are monitored.

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Some specific features of chip electrophoresis with column-coupling in ultra(trace) analysis

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This presentation is intended to overview key aspects of capillary electrophoresis (CE) performed on a column-coupling (CC) chip. Various approaches which may enhance an analytical utility of the CC chip, especially, in ultra-trace analysis will be shown as well. Currently, CE chips provided with CC technology can be considered as multifunctional CE devices designated for fundamental electrophoresis methods (CZE, ITP, IEF) and also for their on-line combinations (ITP-ITP, ITP-CZE and CZE-CZE). Such devices include all relevant analytical functions, e.g. sample clean-up, pre-concentration, separation and detection of the analytes [1-5].

This contribution will show some specific CE tools and analytical benefits of the CC chip. A main attention will be paid on following topics:
(1) Transferring CC-CE to chip electrophoresis. Some methodological aspects
(2) Analytical benefits of ITP-CZE on the CC chip in ultra(trace) analysis
(3) An electrophoretic counter-flow on the chip. Eliminating an undesirable transfer between the coupled columns in ITP-CZE [6]
(4) A transfer of the membrane extraction to the chip electrophoresis. A combination of CC-CE chip with supported liquid membrane extraction on a PP membrane

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Monolithic platform for analysis of therapeutic antibodies

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Antibodies are one of the most useful tools in clinical immunological and biochemical laboratories for many years. Antibody-based therapies using both polyclonal and monoclonal antibodies are emerging as a powerful therapeutic approach enabling treatment of a variety of diseases. However, extensive and complex analytical processes are required to generate well-characterized biologics. The characterization of primary structure and posttranslational modification such as glycosylation is also an integral part of the quality control and quality assurance of biotechnology products.

Capillary microreactors containing trypsin, endoproteinase LysC, and PNGase F immobilized on a porous polymer monolith have been prepared and used for the characterization high molecular weight human immunoglobulin G. One of the important issues we had to deal with was non-specific adsorption of proteins and peptides on the monolithic support. The hydrophilicity of diol functionalities originating from the hydrolyzed poly(glycidyl methacrylate-co-ethylene dimethacrylate) monolith was found not to be sufficient to avoid adsorption of hydrophobic albumin in highly aqueous mobile phase. Therefore, pores of this monolith were first hydrophilized using photografting of poly(ethylene glycol) methacrylate followed by photografting of a 4-vinyl-2,2-dimethylazlactone to provide the hydrophilic pore surface with reactive functionalities required for immobilization. This new approach significantly reduced the undesired non-specific adsorption and facilitated control of both enzyme immobilization and protein treatment processes.

The capillary reactors with immobilized trypsin and endoproteinase LysC were coupled with MALDI/TOF-MS, ESI/TOF-MS, and/or integrated into a multidimensional system comprising monolithic capillary enzyme reactor, in-line poly(lauryl methacrylate-co-ethylene dimethacrylate) monolithic nanoLC column for the separation of peptides, and ESI/TOF-MS for characterization of molecular mass enabling assessing primary structure of the protein. The characterization of glycosylation of immunoglobulins G was achieved via analysis of glycans released from the protein after using PNGase F immobilized on the monolithic support followed by mass spectrometric identification.

Shedding LED light on synergies between analytical science, miniaturisation, photochemistry, and photonics

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Miniaturisation was an exciting novelty in chemistry during the 1990s and since then has become well established in all science and engineering disciplines. It has had an immense influence on providing areas for and the acceptance of multidisciplinary research leading to the convergence of sciences and engineering. Furthermore, advances in photonics, largely driven by the needs of lucrative IT and consumer electronics industries, have benefited other areas including chemistry. Analytical science can profit from these synergies of different areas if they are brought together to produce new concepts and approaches. This presentation will illustrate some of these synergies using a few examples from the recent work in the speaker's research group. These include using light emitting diodes (LEDs) as light sources: (i) new photo-initiated polymerisation of monoliths using LEDs in UV and visible spectral range including photoplymerisation of monoliths by red LED light in polyimide coated capillaries [1] and (ii) development of new detection technologies involving LEDs and specifically of a combined one-point-of-detection '3-in-1' photometric, fluorimetric and contactless conductometric on-capillary detector for CE and other capillary separation techniques [2, 3].

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Acknowledgements

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Electromigration and molecular biology methods in determination and identification of clinical strains of pathogenic bacteria

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In modern clinical laboratory and medical diagnosis a very important problem is searching the new biomarkers of disease state. In medicine, a biomarker can be a substance whose detection indicates a particular disease state (for example, the presence of an antibody or characteristic species of bacteria may indicate an infection). The complex nature of biological samples as well as the low concentrations of analytes require a system with high sensitivity and efficiency. Such requirements provides capillary zone electrophoresis (CZE) which is a powerful and versatile separation technique and can be very useful in analysis of small particles of colloidal sizes and in particular cells.

Electrophoretic separation of bacteria has gained the interest of several research groups. Although the idea of the electrophoretic movement and separation of the cells might seem obvious and simple, the realization of it turns out to be not easy and straightforward. The main problems are the undesired phenomena such as aggregation (cluster formation) and adhesion of the bacterial cells to the inner capillary wall which are consequences of natural behavior of such analytes. Different attempts were made to more or less control the separated microorganisms and focus them into sharp zones which give the analytical signal (peak).

The bacterial pathogen *Staphylococcus aureus* is responsible for a significant amount of human morbidity and mortality. Novel methods, based on CZE and molecular analysis of a part of the *coag* gene were designed for the identification and the differentiation of three *S. aureus* strains. To perform coagulase gene typing, the repeated units encoding hypervariable regions of the *S. aureus* gene were amplified by polymerase chain reaction (PCR) technique followed by restriction enzymes digestion and analysis of restriction fragment length polymorphism (RFLP) patterns as well as sequencing. Proposed procedures, specially fast and cheap CZE, with molecular analyses as the confirmation of these results, could become an effective tool for diagnosis of certain diseases caused by different strains of *S. aureus*. Finally, the results of electrophoretic measurements with molecular analysis were compared. The results presented in this report give a sufficient and real grounds to conclude that CZE could be a novel, fast and cheap method of identification and typing of bacterial strains. However, future investigation are necessary to improve this method and create database.

Acknowledgements .
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HILIC type selectivity in LC – HYPE or HOPE

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Hydrophilic Interaction Chromatography, HILIC, became increasingly popular in the last years as the need for chromatographic methods being able to cope with highly polar compounds increased. This trend was driven by life science applications and other analytical problems as well. The underlying retention and selectivity causing mechanisms of HILIC are still debated but accumulating evidence suggests that the molecular distinction process is often not only based on partitioning of the solutes between a polar modifier-rich (usually water) layer on the sorbent surface but also by adsorption interactions with the sorbent surface per se or polar ligand functionalities attached to it. This implies that not only weak intermolecular interactions between sites of the adsorbent and the analytes can come into play (e.g. hydrogen bonding) but also strong forces (ion attraction, ion repulsion) in case of ionizable functionalities. Both may contribute to retention thus leading to a "mixed mode" mechanism.

With other words, HILIC as general term refers in the first place to mobile phase conditions being rich on an organic solvent (preferably acetonitrile) and low in water or other polar protic modifier content, secondly it refers to polar stationary phases (partially) featuring an adsorbed water or polar protic solvent layer, and thirdly, one needs to specify possible electrostatic interactions tracing back to the charge situation of the adsorbent and of the investigated analytes as function of the mobile phase pH and buffer conditions. Thus, the HILIC methodology is orthogonal to the well known RP system.

In the light of representative examples we will try to highlight the potential of HILIC but also to shade more light on the rather complex retention and selectivity principles which in turn bear intrinsic limitations of the method.

Fast and sensitive detection methods for endotoxin analysis in microchips

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Bacterial endotoxins (lipopolysaccharides, LPSs) are physiologically active components of the outer membrane of gram-negative bacteria and are released during growth, division and lysis. They have been recognized as the most potent stimulants of mammalian immune systems, causing a wide spectrum of pyrogenic and toxic reactions. LPS consists of a lipid region, termed lipid-A covalently attached to a polysaccharide region. Both regions have extremely high variability in their structures, which directly affects their physiological impact. Though several methods

have been used for endotoxin analysis, much progress is still needed to separate and identify the many subclasses and clarify the structure-function relationship of LPSs from individual strains. The lack of strongly UV-active groups or chromophores in the LPS molecule and its strong tendency to aggregate in aqueous solution makes the detection of the underivatized substances difficult.

A comprehensive study was continued to explore the complex structure of the components with several unique sugar components and differences in the lipid part. The novel and fast methods using conventional capillaries and microchips with LIF detection developed especially for endotoxins allowed us 1) to differentiate between R and S endotoxins, 2) to monitor endotoxin-protein complexes and 3) determine the molecular components of the toxic variants of LPSs. MALDI-TOF MS, GC-MS and CE-MS studies were conducted to prove the presence of the different molecular forms, including the "absolute R", this form together with the "core", which contained unusual heptose units, and also the repeating units that are responsible for toxicity and immunogenicity. The techniques developed are usable to analyse and confirm the structures and types of LPSs directly from the cell cultures of the bacteria. This is of high importance, when fast analyses are necessary in infection and in preparation of human vaccines.

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Sample preparation issues in complex carbohydrate analysis by capillary gel electrophoresis

András Guttman and Marcell Olajos
Horváth Laboratory of Bioseparation Sciences, University of Innsbruck, Austria

This presentation gives a thorough evaluation of various sample preparation methods for multicapillary gel electrophoresis based glycan analysis to support electokinetic injection. First the removal of excess derivatization reagent is discussed. While the Sephadex G10 filled multiscreen 96 well filter plate and Sephadex G10 filled pipette tips enabled increased analysis sensitivity, polyamide DPA-6S pipette tips worked in particularly well. In this latter case an automated liquid handling system was used to increase purification throughput, necessary to feed the multicapillary electrophoresis unit. Problems associated with the high glucose content of such biological samples as normal human plasma were solved by applying ultrafiltration. Finally, a volatile buffer system was developed for exoglycosidase based carbohydrate analysis.

Process analytical technology – new opportunities for pharma and related industries

Martin Polčík, ANF DATA (Siemens), Londýnské náměstí 4, 639 00 Brno

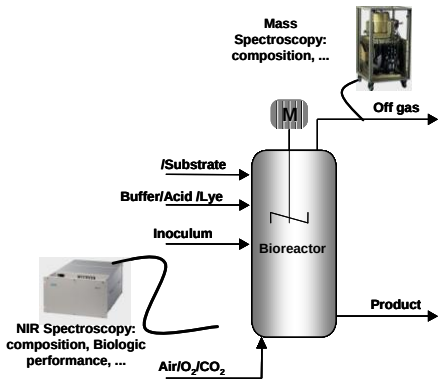
Based on the recent FDA regulations, the so called Process Analytical Technology (PAT) offers the pharmaceutical industry the possibility to increase product quality consistence and to reduce product risks through:

- increased process knowledge and understanding
- optimized process control,

by the use of process analytical technology tools.
These tools are:

- process analyzers
- multivariate tools for design, data acquisition and analysis
- process control tools
- continuous improvement and knowledge management tools.

As schematically shown in the Figure, process analyzers are instruments , such as near infrared spectrometers, fluorescence sensors, acoustic emission spectrometers etc, which provide information about the process. The outgoing on-line data are analyzed using multivariate tools to create quantitative fingerprints of the process by which the quality of the resulting product can be monitored. The identified critical parameters are then kept at their proper values by sending feedback to the control. The aim of this approach is to introduce the so called quality by design (QbD) as an opposite to the quality testing of the resulting product. This recent approach can bring reasonable benefits to the users by speeding up the manufacturing process, limiting the necessity of storing the intermediate products, scaling up the processes and assure fast transfer of the R&D results to the manufacturing.



Siemens as an innovative company provides tools to implement PAT. The SIPAT system makes it possible to synchronize data from different sources of data – instruments, files and process data systems. The collected data can be analyzed to characterize the quality of the product and/or they can be used as feedback to the process control system. Examples of the application of SIPAT will be presented. Also some opportunities for the research will be discussed.

Project of the Central European Synchrotron Laboratory

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Institute of Scientific Instruments of the ASCR, v.v.i., Brno

The CESLAB synchrotron is a third generation synchrotron radiation facility that has been designed with several objectives in mind:

- The usable range of photon energies should extend to at least 40 keV.
- The photon beam should have high stability and a lifetime longer than 10 hours.
- The accelerator should incorporate many straight sections for a variety of insertion devices.
- The dimension of the source at the extraction points should be, at most, 0.25 mm.
- The light source should have a vertical collimation comparable to that of the natural SL emission.

The above considerations have led us to an accelerator energy of 3 GeV and a magnetic lattice that is essentially an Expanded Double Band Achromat.

The main facility will be based on the latest 3 GeV European synchrotron ALBA, currently under construction. The knowledge transfer, help and direct collaboration on the project planning and later on synchrotron construction has been agreed with the experienced team in ALBA with a support by the respective Czech and Spanish ministries. From a technical point of view, the storage ring of diameter 270 m will consist of 24 straight sections for insertion devices for up to 33 beamlines (Fig.1).

Fig. 1 Architectural view of the CESLAB facility

Beamlines are the heart of results at the synchrotron facility. They provide necessary equipment for the methods applied to different fields of research. They were proposed to support research in biology and medicine, material science, chemistry, microtechnology and nanotechnology, environmental sciences, archeology and other disciplines. The methods of X-ray scattering (high-resolution diffraction, powder diffraction and grazing/small angle scattering), crystallography (single crystals, macromolecules), spectroscopy (absorption, Mössbauer), and imaging (absorption, phase-contrast and coherent, diffraction) will be available at dedicated beamlines. A multipurpose Xray optics beamline will be available for generic applications,

including testing of new components, methods and for metrology. While most of the beamlines will work in the X-ray region, a beamline for VUV chemistry in gas phase and two IR stations for spectroscopy and ellipsometry are proposed as well.

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About the invited speakers

Daniel R. Knapp completed his Ph.D. in organic chemistry at Indiana University in 1969. Following postdoctoral work in organic chemistry at the University of California, Berkeley, he moved to the University of Cincinnati Medical School where he was appointed as an Assistant Professor of Experimental Medicine. He moved to the Medical University of South Carolina in 1972 where he now holds the title of Distinguished University Professor and Director of the Proteomics Center. He is also Director of the MUSC Cardiovascular Proteomics Center, one of ten proteomics centers supported by the US National Heart, Lung and Blood Institute. His research interests have centered around biomolecular mass spectrometry with his current activity primarily in development of multi-ionization platforms and microfluidic devices for proteomic analysis. He is the author of the book *Analytical Derivatization Reactions* published by Wiley Interscience.

Holger Becker is co-founder and CSO of microfluidic ChipShop GmbH. He obtained physics degrees from the University of Western Australia/Perth and the University of Heidelberg. He started to work on miniaturized systems for chemical analysis during his PhD-thesis at Heidelberg university, where he obtained his PhD in 1995. Between 1995 and 1997 he was a Research Associate at Imperial College. In 1998 he joined Jenoptik Mikrotechnik GmbH. Since then, he founded and led several companies in the field of microsystem technologies in medicine and the life sciences. He leads the Industry Group of the German Physical Society and is member of the program committee of the SPIE "Microfluidics, BioMEMS and Medical Microsystems" conference as well as acting as regular reviewer of project proposals on national and EU level and for several journals devoted to microsystem technologies.

Jörg P. Kutter received his B.S in chemistry in 1991, and his Ph. D. in analytical chemistry in 1995, both from the University of Ulm, Germany. Both theses focused on chromatographic and electrophoretic separation techniques. After graduation, he was a postdoctoral research fellow in the Laser Spectroscopy and Microinstrumentation Group at Oak Ridge National Laboratory (Oak Ridge, TN, USA) developing microchip-based analytical tools. In June 1998, he joined the Department of Micro and Nanotechnology (formerly, MIC) of the Technical University of Denmark (DTU) in Lyngby, Denmark. In 2006, he was appointed professor in experimental lab-on-a-chip systems at DTU. He is group leader of the internal ChemLabChip Group focusing on the development of microfluidic devices for applications in life sciences. He is also head of the LabChip section of the department.
Dr. Kutter has extensive experience in leading scientific projects, has supervised and co-supervised 18 PhD students, has more than 60 international peer-reviewed publications and books/book chapters and is involved in several international conference committees and professional organizations.

Pavel Neuzil received his Ph.D. degree in electrical engineering from the Czech Technical University in Prague, Czech Republic. He was a postdoctoral fellow at the

University of Illinois, research associate at Stanford University and a principal research scientist at the Institute of Bioengineering and Nanotechnology, A-STAR, Singapore. He is currently a Member of Technical Staff at the Institute of microelectronics, Singapore as well as adj. associate professor at the EEE department, NTU. His main research interests are developing new and simple methods and systems for LOC applications, tools for diagnostic such as fast drug screening systems, optoelectronics and MEMS as well as nature-inspired nanosystems, for example using geckos and lotuses.

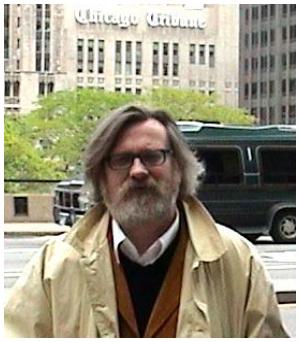
Alejandro Cifuentes is a Full Research Professor at the Institute of Industrial Fermentations in Madrid, Spain. He is the Acting Director of the Institute of Food Science and Deputy Director of Institute of Industrial Fermentations. Since November 2007 he has also been appointed Associated Professor by the University Autonoma of Madrid. He has published more than 150 SCI papers and is currently member of the Editorial Board of 9 international journals. He has collaborated as Guest Editor for Electrophoresis, Journal of Pharmaceutical and Biomedical Analysis, J. Chromatogr. A and Journal of Separation Science on special issues devoted to Food Analysis, Bioactive compounds and Nutrigenomics.

Vladimír Havlíček is the Head of Laboratory of Molecular Structure Characterization at the Institute of Microbiology, Academy of Sciences of the Czech Republic in Prague. He has published more than 80 papers, has more than 500 citations and Hirsch index 15. He received multiple national awards (Learned Society of Czech Society, Otto Wichterle Premium from the Academy of Sciences of the Czech Republic, Bader Prize, etc.). He is a deputy in the Academic Congress (Academy of Sciences of the Czech Republic), member of the European Academy of Sciences and Arts, member of the Scientific Council of the Institute of Molecular Genetics, Prague, editor of the European Journal of Mass Spectrometry and advisory board member of the Journal of Mass Spectrometry.

Jiří Homola (MS 1988, PHD 1993) is Head of Photonics Division and Chairman of Department of Optical Sensors at the Institute of Photonics and Electronics, Prague (Czech Republic). He also is Affiliate Associate Professor at the University of Washington, Seattle (USA). His research interests are in photonics and biophotonics with emphasis on optical sensors and biosensors. J. Homola is a member of Editorial Board of Sensors and Actuators B (Elsevier) and Associate Editor of Journal of Sensors (Hindawi). J. Homola is a member of Permanent Steering Committees of Advanced Study Course on Optical Chemical Sensors and Europt(r)ode Conference Series. He is also member of NATO CBP Advisory Panel. In 2006 J. Homola received Roche Diagnostics Prize for Sensor Technology. J. Homola holds 2 patents, edited 2 books, 6 book chapters, over 70 papers in peer-reviewed scientific journals and presented over 150 research papers at international conferences.

Sven Preuss graduated from the University of Heidelberg, Germany, in 2001 with a Diploma thesis in the field of high energy astrophysics. He then joined Heidelberg Instruments Mikrotechnik to work on spatial light modulators for laser lithography systems and received his Ph.D. in physics for this work in 2004. He returned to Heidelberg Instruments to work in technical sales and is now responsible for the European business development.

Staffan Nilsson received his doctorate in Medical Science (PhD), in 1988 and



currecntly is a professor of Pure & Applied Biochemistry at LTH, Lund, Sweden. He has a very broad range of interests including: Membrane Proteins; Protein Chromatography; Adipocytes; single cell-studies, Cell-cell communication; Adipocytes/pancreatic ß-cells; Cell-entrapment; Self-contained analysis system (hCG, C-reactive protein); Chip µ-Field-Flow Fractionation; CE, peptide-, chiral separation, interaction protein-drug molecule, Capillary Affinity Gel Electrophoresis (CAGE); monolithic-CEC; Laser induced Fluorescence Imaging Detection; Miniaturized analysis systems; Molecular imprinting (MIP) combined with CEC. Cell-based screening systems; Nano-chemistry in levitated droplets; Nanoparticle-based CEC combined with ns-ESI-MS. Staffan has authored over 100 scientific works including 8 book chapters, 3 popular publications (Swedish), 5 patents and 4 patent applications and is one of the frequently invited speakers at international conferences.

Dušan Kaniansky , born in Ráztočno (Slovakia), graduated from Comenius University in Bratislava, 1971. He obtained his Ph.D. in the field of capillary isotachophoresis and D.Sc. in capillary electrophoresis as covering various electroseparation analytical techniques and instrumentation. Professor of Analytical Chemistry at Comenius University, he is currently leading the Department of Analytical Chemistry of this university. He has published well over 100 scientific papers and more than 20 patents as covering different topics in capillary electrophoresis (CE) and electrophoresis on chips (a-lab-on-a-chip). Of these should be noted, e.g., capillary isotachophoresis (ITP), capillary zone electrophoresis (CZE), ITP-CZE and CZE-CZE combinations. In this context, he contributed significantly for developments of instrumentation for CE separation systems. In addition, introduced and/or contributed to the detection techniques for ITP and CZE (conductivity, photometric, radiometric and amperometric CE detectors; together with Ernst Kenndler, co-introduced an off-line combination ITP-MS).

Jana Křenková studied Analysis of Biological Materials at the University of Pardubice, Czech Republic, where she graduated in 2003. The same year, she joined the Department of Bioanalytical Instrumentation, Institute of Analytical Chemistry of the ASCR, Brno and in 2007 she received Ph.D. degree in analytical chemistry from the University of Pardubice, working on the development of capillary enzyme reactors for integration into microfluidic systems. Currently she is a postdoctoral fellow at the Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, USA in the group of Dr. Frantisek Svec. The aim of her research is focused on the development of monolithic platform for analysis of therapeutic antibodies.

Mirek Macka, after completing MSc in chemistry at Masaryk University Brno 1981, worked as research scientist in pharmaceutical industry in Czechoslovakia and later in Switzerland, conducting analytical research, method development and validation. In 1994 he made a career move to academia and in 1997 completed PhD in Chemistry at the University of Tasmania, Australia, in the area of capillary electrophoresis. Following a Research Fellowship at the University of Tasmania till 1999, in 2000 he was awarded a 5-year prestigious mid-career fellowship "Australian Research Council Australian Research Fellowhip". In 2005 he received another highly competitive and prestigious award - the EU funded senior level fellowship "Marie Curie Excellence Grants", which he took up from 2006 as a Marie Currie Fellow and Excellence Team Leader at Dublin City University, Ireland, working in the area of miniaturised and microfluidic systems in chemical analysis and chemistry. His diverse research interests include fundamental and instrumental aspects of capillary electrophoresis including optical and electrochemical detection, utilization of light emitting diodes for optical detection systems and as light sources for photoinitiated polymerizations and photopatterning of monoliths, design of unusual 'exotic' monoliths for separation science, and most recently microphotochemistry. He is co-founder of Separation Science Cluster, a multidisciplinary collaborative research unit at DCU, and co-PI on Irish Separation Science Cluster initiative.

Ewa Kłodzińska successfully completed her PhD thesis in 2007 focusing on new procedure of separation and identification of microorganisms by capillary electrophoresis for medical diagnosis. Now she works as an assistant at the Chair of Environmental Chemistry and Bioanalytics headed by Prof. Bogusław Buszewski. Her researches are related to miniaturization of separation systems including micro- and nano - capillary columns and to preparation of different stationary phases with specific properties dedicated for these groups of compounds. She obtained very satisfying and interesting results for separation of five different species (*E. coli*, B. cereus, *Arthrobacter g*., *Pseudomonas sp*., Micrococcus sp.). The main goal of her studies and researches was to resolve the same species of bacteria in the range of different strains (*S. aureus*, *H. pylori, E. coli*) and to propose the rapid method for identification of those pathogenic bacteria. Actually, she is involving in studies based on two-dimensional electrophoresis (2 D PAGE) as a tool for separation and determination of proteins from microorganisms in samples of cancerous tissues. On the other hand, together with microbiologist, she starts to work with molecular biology methods (PCR, DGGE) for differentiation of pathogenic bacterial strains.

Wolfgang F. Lindner was appointed 1996 for a Chair of Analytical Chemistry at the University of Vienna. Already during his studies his research interest was influenced by pharmaceutical (life) sciences and by separation sciences related to HPLC, GC, CE/CEC and MS. In this context particular interest developed in non-covalent interactions and molecular recognition with focus on stereochemistry and enantiomer discrimination. The development of novel synthetic selectors (receptors) useful for enantioselective separation techniques lies at the interface of organic, analytical and biological chemistry which characterizes best his scientific credo. He was trained in Organic Chemistry at the University of Graz (Austria) receiving his PhD in 1972.

Then he moved on to the Institute of Pharmaceutical Chemistry in Graz specializing in pharmaceutical analysis. In 1978 he was awarded a Max Kade postdoctoral fellowship which he held at North Eastern University in Boston (USA) under the supervision of Prof. Barry L. Karger. In 1986 he was visiting scientist at the FDA/NIH in Bethesda (USA) where he got exposed to biological chemistry, a subject his research group is approaching from various angles.

Prof. Lindner was editor of Journal of Chromatography B from 1995 to 2006 and is serving as editorial/advisory board member for Chirality; Chromatographia; J. Pharmac. Biomed. Anal.; LC-GC International; J. Chromatogr. B, J. Biochromatogr.; J. Analytical and Bioanalytical Chemistry; Current Analytical Chemistry.

He has published more than 330 scientific publications, 12 book articles, holds several patents and is active in educational programs for diploma and PhD student as well as for post doctoral fellows.

Ferenc Kilár finished his studies in 1977 at Eötvös Loránd University, Budapest. After finishing his university studies he was working at the Institute of Enzymology, Budapest, and then he moved to Pécs, where he is working at the University of Pécs since 1983. He received his PhD (CSc) in 1986 and the degree of Doctor of Science in 1995. He was a visiting researcher more than 5 years in Uppsala, Sweden at the Department of Biochemistry, working on the development and application of capillary electrophoresis, mainly using this technique in protein research. In 1997 he was appointed to be a full professor and since then he is the Head of the Department of Analytical Chemistry and director of the Institute of Bioanalysis. Since 2000 he is the Head of the Doctoral School in Chemistry at the University of Pécs. His main research area covers protein-chemistry and the development and application of modern separation methods in bioanalysis. He is a co-author of more then 100 scientific publications and 3 books. He is a member of several national and international research consortia and received several national and European grants for his research with a sum of more than 800000 €. He was a visiting professor at Universitá "La Sapienza" and Istituto di Cromatografia, Rome, Italy, University of Bern, Switzerland and L'Institut Pasteur, Paris, France. He is the member of the editorial boards of Journal of Biochemical and Biophysical Methods (2001-2008), Hungarian Chemical Journal (2001-2007), Studia Universitatis Babes-Bolyai Chemia (since 2007), Electrophoresis (since 2008), Journal of Proteomics (since 2008).

András Guttman heads the Horváth Laboratory of Bioseparation Sciences (HLBS) at University of Innsbruck (Austria). His main research interests are affinity based separation materials, quantitative proteomics, microfabricated device technology and biomarker discovery. Dr. Guttman held positions previously at Novartis (La Jolla, CA), Genetic Biosystems (San Diego, CA) and Beckman Instruments (Fulleton, CA). He has more than 220 scientific publications including 29 book chapters, edited several textbooks and holds 15 patents. He is an associate director of CASSS and on the editorial boards of numerous international scientific journals. Dr. Guttman graduated from the University of Veszprem (Hungary) in chemical engineering, where he also received his Ph.D. He was awarded the Analytical Chemistry Award of the Hungarian Chemical Society in 2000 and became a member of the Hungarian Academy of Sciences in 2004.

Martin Polčík has been with Siemens (ANF DATA) since 2004 where he works as an consultant. He has been involved in various R&D projects mainly in the Life Science area. Before that he worked at the Fritz-Haber-Institut der MPG on the applications of synchrotron radiation. He is an co-author of more than 70 papers mainly in surface physics, surface chemistry, atomic force microscopy and more recently bioinformatics.

Posters

Standard systems for measurement of pKs and ionic mobilities. 1. Univalent weak acids

Šlampová A., Křivánková L., Gebauer P., Boček P.*
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Determination of pK values of weak bases and acids by CZE has already attracted big attention in current practice and proved to offer the advantage of being applicable for mixtures of analytes. The method is based on the measurement of mobility curves plotting the effective mobility vs. the pH of the background electrolyte, and following computer-assisted regression involving corrections for ionic strength and temperature. To cover the necessary range of pH for a given case, both buffering weak acids and bases are used in one set of measurements, which requires implementing computations of individual ionic strength corrections for each pH value. It is also well known that some components of frequently used background electrolytes may interact with the analytes measured, on forming associates or complexes. This obviously deteriorates the reliability of the resulting data. This contribution brings a rational approach to this problem and establishes a standard system of anionic buffers for measurements of pKs and mobilities of weak acids, where the only counter cation present (besides H^+) is Na^+. In this way, the risk of formation of complexes or associates of analytes with counter ions is strongly reduced. Moreover, the standard system of anionic buffers is selected in such a way that it provides, for an entire set of measurements, constant and accurately known ionic strength and the operational conditions are selected so that they provide constant Joule heating. Due to these precautions only one correction for ionic strength and temperature is needed for the obtained set of experimental data. This considerably facilitates their evaluation and regression analysis as the corrections need not be implemented in the computation software. The reliability and the advantages of the proposed system are well documented by experiments, where the known problematic group of phenol derivatives was measured with high accuracy and without any notice of anomalous behaviour.

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Standard systems for measurement of p*K*s and ionic mobilities. 2. Univalent weak bases

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This contribution presents a standard system of cationic buffers for measurements of p*K*s and mobilities of weak bases by capillary zone electrophoresis. The system is designed so that all buffers comprise the same concentration of Cl^- present as the only counter anion. This minimizes problems caused by interactions between the counterion and the analytes which may otherwise bring biased values of obtained effective mobilities. Further, the buffer system provides constant and accurately known ionic strength for an entire set of measurements. When additionally all measurements are performed with constant Joule heating, one correction for ionic strength and temperature is then needed for the obtained set of experimental data. This considerably facilitates their evaluation and regression analysis as the corrections for ionic strength and Joule heating need not be implemented in the computation software and may be applied only once to the final regression results. The reliability and the advantages of the proposed system are well documented by experiments, where the known problematic group of amines and pyridine were measured with high accuracy and without any notice of anomalous behavior.

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Considerations on selected factors influencing preparation and utilization of polymeric monolithic capillary columns

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Monolithic columns have attended a great attention of researchers because of easy way of preparation, possibility of tailoring of the porosity as well as surface characteristics. A wide spectrum of available monomers allows obtaining many different stationary phases of desired chromatographic properties.

The general procedure of preparation of the polymeric monoliths includes:

- modification of the capillary wall;
- preparation of the polymerization mixture;
- filling of the capillary;
- polymerization;
- flushing to get rid of unreacted compounds.

The modification of the capillary wall is the initial but important step in the preparation of the monolith, the result of which is covalent attachment of the monolith to the capillary wall to increase the stability and mechanical strength of such a stationary phase. Here we discuss several of the procedures of capillary wall modification which can be found in the literature. The comparison is based on the data obtained from the wetting angle measurements, adhesion test and XPS data.

It is known that there are two main synthetic paths of monolithic columns preparation – thermal polymerization and photopolymerization. A great advantage of the latter method is much faster polymerization process which typically takes 1-2 hours, in comparison to 16-24 hours of thermal process. Our results show that temperature control during the photopolymerization can have enormous influence o the monolithic column properties.
The utilization of the monolithic capillary columns in CEC is possible if only sufficient electroosmosis can be generated by a stationary phase. It is generally required that the CEC stationary phase contains charged functionalities to provide electric double layer creation and thus fast EOF. However, it was found that it is possible to obtain electroosmosis on neutral (e.g. polymeric) surfaces. In our previous work we observed very fast EOF on DVB modified capillaries and we extended our investigation to monolithic polystyrene-based neutral monolithic capillary columns. In this contribution we present our recent results on the study of EOF on such stationary phases.

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Modeling of the effect of separation temperature and organic modifier concentration in capillary zone electrophoresis of tripeptides

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This work was focused on investigating the effects of two separation influencing parameters, temperature and organic solvent concentration, upon electrophoretic migration in capillary zone electrophoresis (CZE). Various two variable semi-empirical models and back-propagation artificial neural networks (BP-ANN) were applied to predict the electrophoretic mobilities of model peptides with non-polar, polar, positively charged, negatively charged and aromatic R group characteristics. Previously published work on the subject did not account for the influence of temperature and buffer organic modifier concentrations on peptide mobility,in spite of the fact that both were considered to be influential factors in peptide analysis. In this work, a substantial data set consisting of measured electrophoretic mobilities in 30 mM PO4 buffer at pH 7.5, at 20, 25, 30, 35 and 40°C and at five different organic additive containing running buffers (0, 5, 10, 15 and 20% MeOH) as well as two applied electric field strengths (12, 16 kV) was built up for the evaluation of our mobility predicting models. Arrhenius plots of natural logarithm of model peptides mobility vs. reciprocal absolute temperature for the various parameter setups as well as their activation energies were investigated and compared. A complex impact of both temperature and organic additive concentration on the electrophoretic mobilities of the model peptides was found and systematically evaluated. Calculated mobilites by two variable semi-empirical and BP-ANN models were compared to each other and to the experimental data, respectively. Neural network approaches were able to model the complex impact of both temperature and organic solvent concentration and achieved considerable higher predictive power than the two variable semi-empirical models supporting the assumptions on a non-linear structure-mobility relationship of the investigated peptides.

Detection of tetrodotoxin (TTX) using a surface plasmon resonance (SPR) biosensor

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Tetrodotoxin (TTX) is a potent low-molecular weight (~319 Da) marine neurotoxin found in a range of organisms including certain species of puffer fish and amphibians.
Traditional screening methods for TTX include mouse bioassay, high performance liquid chromatography, mass spectrometry, and enzyme-linked immunosorbent assay. However, these methods are rather laborious and time consuming.

In this contribution, we present a surface plasmon resonance (SPR) biosensor for rapid detection of TTX. SPR biosensors are optical biosensors which measure refractive index

changes induced by the binding of analyte to its biospecific partner (antibody) [1]. In this study, a spectral SPR sensor developed at the Institute of Photonics and Electronics, Prague and binding inhibition detection format were used. In this detection format, sample with an unknown concentration of TTX was incubated with a known concentration of a-TTX antibody and then the unreacted antibody was detected by the SPR sensor coated with TTX. TTX was immobilized on the SPR sensor surface via a mixed self-assembled monolayer consisting of hydroxyl terminated oligo-ethylene glycol (OEG) alkanethiol (OH-OEG-AT) and amine terminated OEG alkanethiol (NH_2-OEG-AT). TTX was covalently coupled to the amine groups on the NH_2-OEG-AT via formaldehyde reaction [2]. In the first stage of TTX detection experiments, detection of known concentrations of TTX was performed to establish a calibration curve. Then, the sensor was used to measure TTX in unknown samples (PBS, 10% or 100% puffer fish matrix) and Boltzmann model of the calibration curve was used to calculate the concentration of TTX in the unknown samples. The limit of detection was determined to be 1.6 ng/ml.

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Temperature effects in boronic acid - lectin affinity chromatography (BLAC) of proteins

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Glycosylation is one of the most important co- and post-translational modification of proteins, playing important roles in many biochemical processes including cellular and molecular communication. Analysis of the human serum glycoproteome concerning disease related glycosilation alterations is of great recent interest, particularly in biomarker discovery. Selective, high throughput glycoprotein enrichment is a major issue in the analysis of complex biological samples such as human serum. Automated boronic acid lectin affinity chromatography (BLAC) is an excellent tool to isolate and enrich various classes of human serum glycoproteins for downstream processing such as N-linked glycan profiling by capillary gel electrophoresis. In this presentation we evaluate the temperature dependency of glycoaffinity enrichment process through a wide temperature range of 5°C - 70°C. Agarose bound wheat germ agglutinin (WGA) and boronic acid beads were used in the study with protein standards of trypsin inhibitor (boronic acid specificity), ribonuclease B (WGA specificity), lysozyme and myoglobin (negative controls). Glycoprotein enrichment level was determined by HPLC analysis at the different temperatures. Our results suggested ambient temperature to be the best for BLAC based enrichment of human serum glycoproteins. At higher temperatures, non-specific interactions with the agarose carrier prevailed, evidenced by the large amount of myoglobin in the eluate. Albeit, lysozyme was present in most of the elution fractions its amount was apparently inversely proportional with the temperature. At low temperatures, on the other hand, significant decrease was observed in glycoaffinity, probably due to the kinetically controlled elution step.

Combined ‘3-in-1’ photometric, fluorimetric and contactless conductometric on-capillary detector for capillary separations: Applicability for simultaneous detection in CE

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A new design of on-capillary detection combining 3 detection modes - photometric (PD), fluorimetric (FD) and contactless conductivity (C^4D) - in one identical point of detection has been recently introduced, tested and characterised [1, 2]. This design allows a combined ‘3-in-1’ detection in capillary separation techniques (CE, CEC, capillary- and nano-LC). A key advantage is the concurrent availability of the three independent on-column detection techniques in one identical detection point, thus avoiding undesirable shifts in separation times caused by multiple parallel detectors. The per-definition concurrent availability of all the 3 detection modes (PD, FD, C^4D) offers another unique advantage for the analysis of complex samples, where each of the single detection modes alone might yield an incomplete detection of the present analytes. The simultaneous detection of analytes with different chemical and detection properties in one single separation run offers more information from a single run and can provide unexpected, revealing and useful results, especially for unknown real samples. In this work, the applicability of the 3-in-1 detector for detection of various analytes in complex matrices related to a range of different analytical application fields is investigated, including protein digest analysis and fingerprinting, analysis of polysaccharides and applications in food, drug and fine chemical analysis.

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New advances in DNA-protein interaction studies

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DNA – protein binding is among the most frequently studied biomolecular interactions and of high importance in contemporary systems biology research. One interesting aspect of this rapidly developing field is the affinity capture of proteins by specific aptamers of G-quartet forming oligonucleotides. G-quartets are structural motifs formed by guanine-rich sequences commonly occurring in the human genome. In our *in vitro* gene expression studies, sequence variations in the highly guanine-rich promoter region of the dopamine D4 receptor gene apparently acted as enhancing or silencing factors, presumably caused by the different affinity of the polymorphic alleles to the corresponding transcription factors. Therefore, a model system was developed to identify G-quartet forming sequences of interest that were then bound to magnetic beads to form an affinity capture surface for target proteins consequently analyzed by MALDI-TOF-MS. G-quartet formation of different DNA molecules was verified by means of a simple and rapid capillary gel electrophoresis based method. The adaptability of this novel approach for the analysis of G-quartet forming sequences with different lengths was proved with 15-, 19-, 23- and 27-mer oligonucleotides. The applicability of G-quartet forming aptamers in protein affinity capture by oligonucleotide coated magnetic beads was also evaluated. Biotinylated oligonucleotides were immobilized onto streptavidin magnetic beads and used as baits to capture target proteins from biologically relevant samples. Protein capture was demonstrated by direct analysis of the DNA-protein complex at the surface of the magnetic beads by MALDI-TOF-MS. Identification of the captured proteins was performed by mass spectrometric analysis after tryptic digestion. Our approach by applying capillary electrophoresis and magnetic bead based affinity capture combined with MALDI-TOF-MS analysis was apparently suitable to identify G-quartet forming sequences and to analyze the effect of their sequence variations in altering affinity for specific DNA binding proteins.

Isotachophoresis – zone electrophoresis determination of glyphosate in drinking waters on a column-coupling chip

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Glyphosate [N-(phosphonomethyl)glycine] is a non-selective herbicide used on many food and non-food crops. It can be persistent for more than three years in soils and has been found in surface and ground waters. This herbicide is among the most widely

used pesticides by volume. Its usage in 1990 was estimated to be 5259.4 metric tons. In recent years, 13 to 20 million acres in USA were treated with 8 482.2 metric tons annually. The Maximum Contaminant Level has been set at 0.7 ppm (US EPA National Primary Drinking Water Regulations).

The present work was aimed at direct determination of glyphosate in drinking waters using the electrophoresis chip with coupled channels (CC) and a pair of conductivity detectors. The CC chip was provided with two injection channels (0.9 and 9.9 µl volumes) and a 9.3 µl total volume of a pair of the separation channels. To reach a low limit of detection for glyphosate, zone electrophoresis (ZE) with on-line isotachophoresis (ITP) sample pre-concentration and a 9.9 µl volume of the injection channel was favored. ITP separations performed in the first separation channel at low pH (3.2) enabled very favorable sample clean-up from the potential interferences (organic and inorganic acids present in drinking waters), whereas ZE separations performed in the second separation channel at higher pH (6.1) afforded a quick resolution and detection of glyphosate on the CC chip.

Limit of detection for glyphosate under preferred ITP-ZE separation conditions was estimated at a 2.7 µg/l concentration using a 9.9 µl volume of the injected sample. ITP-ZE analysis of model and real samples performed in a hydrodynamically closed separation system with suppressed electroosmotic flow provided very favorable short-term and long-term repeatabilities of the migration (0.1-3.5 % RSD of migration time) and quantitative parameters (0.2-6.9 % RSD of peak area) for glyphosate. Recoveries of glyphosate in spiked drinking waters (10-100 µg/l concentration of glyphosate) varied in the range 99-119 %. Only a minimum pretreatment of drinking water samples (degassing and dilution) and short analysis time (ca. 10 minutes) were characteristic also for ITP-ZE separation of glyphosate on the CC chip with enhanced sample loadability (a 9.9 µl volume of the injection channel).

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Electrophoretic separations of bases on a column-coupling chip

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Adsorption of the complex biological or environmental matrix constituents on the channel walls of the polymer-based chips is a common analytical problem for most microfluidic devices. The interactions between the matrix constituents and chip

surface can cause changes in their migration and quantitative characteristics during electrophoresis separations on the chip. These problems occur also in conventional capillary electrophoresis (CE) with native capillaries made of inorganic or polymer materials. For these reasons, a modification of inner surface of the CE separation systems is required to suppress adsorption of the sample constituents (e.g., proteins, peptides, amines).

In principle, surface modification is realized by two methods: (1) permanent modification and (2) dynamic coating of the chip surface. Permanent surface modification includes specific chemical reactions (e.g., aminolysis, reduction, and photoactivation) which permanently modify the surface of the separation system [1]. Dynamic coating is based on the use of different surface modifiers strongly adsorbed on a surface of the separation channel. Different types of amines introduced to the sample or running buffers were used in this respect [2,3].

This contribution is focused on a study of the adsorption processes on a poly(methyl methacrylate) (PMMA) chip linked with the CZE separations of a group of 13 aliphatic and aromatic mono-, bi- and tri-valence amines. The surface of PMMA chips was modified by dynamic coating (surface modifiers) to prevent an adsorption of studied amines on the walls of chip channels. Surface modifiers as aliphatic oligoamines (diethylenetriamine and triethylenetetramine) were added to the electrolytes which filled the channels on the chip. An impact of an increased modifier concentration on the peak profile of the studied amines has been monitored. CZE experiments were realized in a cationic mode. A propionate carrier electrolyte with an addition of a 100 μmol/l concentration of TETA and a 25 mmol/l concentration of 18-crown-6-ether has been used for the CZE separation of amines, which allowed the separation of 12 from 13 studied amines. Simple dynamic modification of the surface of PMMA chip enabled a fast (up to 10 min.), sensitive and reproducible CZE analysis of the amines.

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Two-dimensional combination of capillary zone electrophoresis with isotachophoresis for complex mixtures

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For the analysis of multicomponent samples e.g. biological samples, one-dimensional separation methods often do not have sufficient selectivity. The two-dimensional (2D) separations provide a greatly enhanced peak capacity (n_c) relative to linear systems, thus substantially reducing peak saturation (m/n_c; where m is actual number of components in the mixture) and the resulting statistical overlap of component peaks from complex samples [1].
This work deals with the possibilities of the 2D approach in a on-line combination of isotachophoresis (ITP) and capillary zone electrophoresis (CZE) in the column-coupling by using of discrete spacers 2D ITP(DS)-CZE. The main goal was to estimate a number of resolved peaks in comparison with common ITP-CZE in anionic separation mode.
In a 2D ITP(DS)-CZE separation mode the sample is injected to the ITP step together with suitable DSs. Four effective DSs were found for the used multicomponent samples (human urine, 59 organic analytes and their mixture). In ITP steady state a separated constituents originated from the multicomponent mixture was concentrated into a five boundary layers (LE/DS1, DS1/DS2,..,DS4/TE). Than only one ITP boundary layer was transferred to the second (CZE) dimension of the separation and a rest of the sample was removed towards a counter electrode of ITP column. Each one of five ITP boundary layers was separated in CZE separately, in a consequent runs. A set of five 2D ITP(DS)-CZE runs provided information about whole multicomponent sample. The separations of multicomponent samples in our cases were not focused on the determination of single analytes but the general purpose was the separation of all components into peaks with total number as high as possible.
In case of all mentioned samples was certified greatly enhanced effectiveness of 2D ITP(DS)-CZE in compare with common ITP-CZE separation, according to count of peaks and their resolution. Beneficial effect of the transport of the analytes to the CZE stage in the separated groups by using of DSs was evident. Comparison of the electropherograms, number of peaks and resolution (urine, model analytes and their mixture) of ITP-CZE and 2D ITP(DS)-CZE shows that 2D systems is efficient tool for increasing of the number of resolved peaks and reduce peak overlap.

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On-line combination of zone electrophoresis with isotachophoresis in a two-dimensional mode

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On-line ITP-CZE combination is mostly used for the separation of analytes which are present in the sample at trace concentration and do not create ITP zones. CZE resolutions of analytes depend (except for separation condition) especially, on the number of analytes which were transferred from ITP to CZE stage. Generally, the peak overlapping increased with increasing number of analytes in CZE stage and resolution of peaks is deteriorated.

In the analysis of multicomponent samples two main problems can be solve: (1) to determine some (trace) analytes present in the sample or (2) to give analytical information about whole sample. In the second case, especially, when a number of analytes is higher than peak capacity of CZE stage, the use of ITP-CZE with a transport of all analytes to CZE is not very effective. ITP-CZE using discrete spacers (DSs) performed in two-dimensional mode (2D ITP(DS)-CZE) and carried out on a column-coupling equipment is a very effective tool to solve this problem. In this mode, the sample is injected to ITP stage together with appropriate DSs. Here, concentrations of DSs in the loaded sample have to be higher as needed for a formation of their fully developed ITP zones and DSs do not give a response for UV detector.

Under ITP steady state conditions, DSs create their own ITP zones and simultaneously, analytes migrate in a "spike" mode in the boundary layers between the ITP zones. Then, only one boundary layer is transferred to the CZE stage, e.g. each boundary layer is separated sequentially in CZE.

The electrophoretic patterns of the individual analyte groups in ITP-CZE and 2D ITP(DS)-CZE separations as well as number of peaks are very similar. The total number of resolved peaks and also their resolution in a five 2D ITP(DS)-CZE experiments was higher than a number of peaks achieved in the ITP-CZE separation of the sample containing all 59 analytes. 2D ITP(DS)-CZE mode is useful in the analysis of multicomponent samples of unknown composition, however different types of samples require an optimization of the DSs.

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System effects in sample self-stacking CZE: Single analyte peak splitting of salt containing samples

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Subject of this contribution is the theoretical and experimental investigation of the electromigration behavior of salt-containing samples in CZE where self-stacking of analytes proceeds due to formation of a transient leading zone with sharp rear boundary by the fast coion of the salt. It is shown that the system may develop in a more complicated way due to the presence of system effects, where the zone of the bulk coion exhibits several transient sharp boundaries capable of stacking analytes. Hence, an analyte present in the original sample may stack simultaneously at mutually different sites which give several peaks for one compound in the detector. Due to the transient character of the above phenomena, the majority of sharp boundaries disappear with time and analytes are destacked. Depending on whether the split analyte zone merges before destacking or not, permanent or transient double or multiple peaks are formed. Simple general explicit rules for the prediction of the behavior of an actual system cannot be given, the approach based on EMD velocity profiles presented in this paper however reveals the phenomena and properties of transient zones and their boundaries. The evolution of an electrophoretic system in question may thus be predicted and analyzed in an exact way.

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Compact device for divergent flow isoelectric focusing

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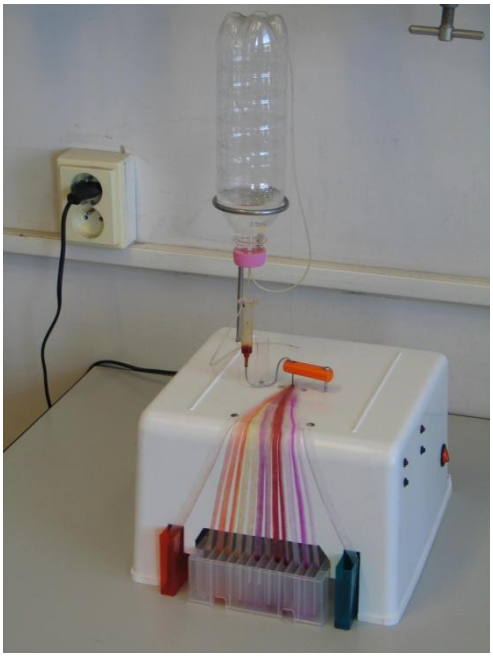
Continuous flow isoelectric focusing (CF IEF) is a process wherein a sample stream is continuously introduced into a carrier ampholyte solution flowing as a thin film. By introducing an electric field perpendicular to the flow direction, ampholytic sample components are separated by IEF according to their differences in pI values.



The solution of current compromises of CF IEF is seen in combination of the features of small separation channels with those of large ones. The basic idea comprises the continuous widening of the flat channel while the liquid flows from channel input toward the outputs which generates a divergent flow and, at the same time, the use small voltage at the channel input and high voltage at the channel output.

The simple instrument for continuous divergent flow IEF (DF IEF) based on our principles outlined previously [1-3] is presented here. The separation and focusing space of a trapezoidal shape is created by a porous bed made from a non woven material with thickness decreasing from narrow input to wide output. The features of device for divergent flow isoelectric focusing are demonstrated using colored synthetic ampholytes.

The essential advantages of the proposed DF IEF device design include its simplicity, efficiency, high throughput and ability to process rough extracts. The separated proteins are obtained in free solution which allows further characterization and identification by various techniques and methods. Used nonwovens are cheap enough to use disposable layers for the separation space. Carriers based on mixture of defined buffers are cheap and advantageous for further treatment of collected fractions. Device performance can be visualized and optimized using colored pI markers.

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Divergent flow isoelectric focusing: Application of the method as a separation and purification step for MS analysis of beer-related proteins

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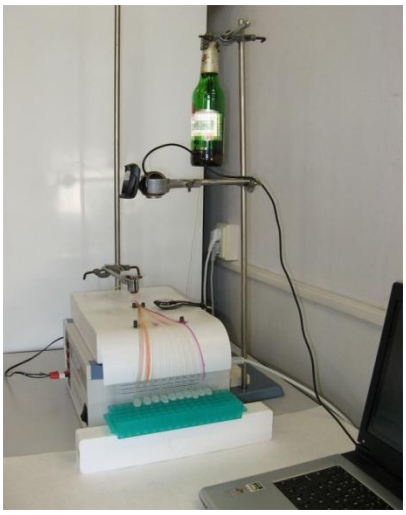
A protein study by MS demands proper purification and separation of complex protein mixtures obtained from biological samples. The method of divergent flow isoelectric focusing (DF IEF) promises improvement of sample preparation in proteomic studies.

DF IEF was carried out in a separation channel with increasing width. The channel was cut out from a polyester non-woven web. DC voltage (800 V) was brought to two pairs of electrodes situated on the channel sides. Amphoteric compounds including proteins drift through the channel carried by flow (18 ml/h) in positions given by their isoelectric points. The pH gradient (3-10) and its stability during analysis have been monitored with colored low-molecular mass p*I* markers. Separated fractions were collected in ten micro vials and further analyzed by MS.



The suggested method was used for separation and purification of crude protein extract from barley grain, malt and beer. Collected fractions of separated proteins were characterized by MALDI-MS. Desalting during IEF enhanced significantly the quality of mass spectra. It also simplified monitoring of post-translational modifications and protein changes occurring during malting and brewing. Results have shown the real potential of the suggested DF IEF lay-out as a high efficient preparative tool for separation and purification of complex protein mixtures for further analyses.

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Development of microfluidic pneumatic nebulizer for electrospray mass spectrometry

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A microdevice combining sample handling (preconcentration, separation, chemical modification, etc.) with electrospray mass spectrometry is of great practical interest in a number of bioanalytical areas. While electrospray is most commonly used in connection with liquid chromatography its integration into microfluidic systems is becoming increasingly popular. Although significant improvements in the efficiency and sensitivity of the analysis have been demonstrated by miniaturization of the separation devices, integration of the electrospray source still requires further development. In this work the glass based microdevices were fabricated by photolithography, wet chemical etching and thermal bonding. A sandwich design with electrode chambers and sample inlet ports located on an external plastic manifold was selected for routine use. Several arrangements of the integrated liquid junction based pneumatic nebulizer were designed for on-line electrospray analysis of peptides. The performance of several experimental micronebulizers was tested with respect to the sample and air flows and electrospray voltages for achieving long term stability electrospray signal.

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Raman microspectroscopy of optically trapped micro- and nanoobjects

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Raman microspectroscopy - the combination of Raman spectroscopy with optical microscopy - allows obtaining spatially resolved maps of the specimen chemical composition. Recording such composition maps becomes challenging for a number of important specimens with sizes in the micron and sub-micron range (e.g. colloidal particles, microdroplets, vesicles, or living cells) that exist normally in suspensions or aerosols and whose function or structure require the presence of the fluid environment. Attempts to apply standard immobilization procedures (e.g. chemical bonding or adsorption to a substrate, micropipette suction) to these specimens often lead to undesired alternation of their properties. Moreover, the presence of a support

substrate can cause a significant spectral background obscuring the observed Raman scattering spectra of the target specimen.

Optical trapping represents an elegant approach for addressing the above mentioned challenge. In its most widely used experimental configuration – optical tweezers - particles with sizes from tens of nanometers to tens of micrometers and refractive index higher than that of the surrounding medium can be confined in the vicinity of the focus of a tightly focused laser beam. Since both optical tweezers and Raman microspectroscopy rely on the use of a strongly-focused laser beam, it is straightforward to combine both techniques in a single experimental setup. This combination has already found numerous applications especially in the fields of analytical and physical chemistry and cell and molecular biology [1]. The combined technique offers the possibility of analyzing individual target microobjects under relevant environmental conditions. Moreover, it allows detecting directly the variations of their individual properties and composition which would be otherwise lost in an ensemble-averaged measurement.

In our contribution, we present a dual-beam system for Raman microspectroscopy of optically trapped micro- and nanoobjects featuring two independent beams for the spectrum recording and optical trapping. We demonstrate the potential of this setup for microanalysis by recording the Raman scattering spectra of individual optically confined micron and sub-micron sized polystyrene beads and baker's yeast cells.

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Temperature effects in boronic acid - lectin affinity chromatography (BLAC) of proteins

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Glycosylation is one of the most important co- and post-translational modification of proteins, playing important roles in many biochemical processes including cellular and molecular communication. Analysis of the human serum glycoproteome concerning disease related glycosilation alterations is of great recent interest, particularly in biomarker discovery. Selective, high throughput glycoprotein enrichment is a major issue in the analysis of complex biological samples such as human serum. Automated boronic acid lectin affinity chromatography (BLAC) is an excellent tool to isolate and enrich various classes of human serum glycoproteins for

downstream processing such as N-linked glycan profiling by capillary gel electrophoresis. In this presentation we evaluate the temperature dependency of glycoaffinity enrichment process through a wide temperature range of 5°C - 70°C. Agarose bound wheat germ agglutinin (WGA) and boronic acid beads were used in the study with protein standards of trypsin inhibitor (boronic acid specificity), ribonuclease B (WGA specificity), lysozyme and myoglobin (negative controls). Glycoprotein enrichment level was determined by HPLC analysis at the different temperatures. Our results suggested ambient temperature to be the best for BLAC based enrichment of human serum glycoproteins. At higher temperatures, non-specific interactions with the agarose carrier prevailed, evidenced by the large amount of myoglobin in the eluate. Albeit, lysozyme was present in most of the elution fractions its amount was apparently inversely proportional with the temperature. At low temperatures, on the other hand, significant decrease was observed in glycoaffinity, probably due to the kinetically controlled elution step.

Combination of NMR and differential scanning calorimetry applied to study thermal denaturation above 100°C

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Lipid transfer protein 1 (LTP1) is a ubiquitous plant protein able to transfer lipids between membranes *in vitro*. Barley LTP1 is a basic protein (pI = 9) consisting of 91 amino acid residues. As other plant LTP1s, its compact structure comprises four α-helices and C-terminal arm stabilized by four disulfide bridges. Barley grain LTP1 contains a lipid-like molecule of 294 Da as a posttranslational modification. Recently, this lipid-like adduct has been identified as a reactive oxylipin (α-ketol 9-hydroxy-10-oxo-12(Z)-octadecenoic acid) produced in barley seeds. Moreover, this protein has a hydrophobic cavity allowing the binding of different types of lipids. In addition, LTP1 is extremely heat and protease resistant, survives all procedure of making beer, and is present in beer and has effect on foaming.

The importance of unusual heat stability of LTP1 was soon recognised. Lindorff-Larsen and Winther investigated stability of LTP1 towards denaturants, proteases, and temperature up to 100°C. In spite of the number of thermal stability studies reported in the literature, we felt that two issues still remained to be addressed in order to understand the impact of the thermal denaturation of LTP1 during brewing and other processes involving heating of barley and other grain products. First, the LTP1 denaturation was systematically studied up to 100°C but higher temperatures are critical for the extent of LTP1 denaturation, as demonstrated e.g. by Van Nierop *et al*. The second and more important issue is the irreversibility of the thermal denaturation of LTP1. While reversible denaturation curves can be interpreted in terms of van´t Hoff enthalphy ΔH and of melting temperature directly related to entropy ΔS of

unfolding ($T_m = \Delta H / \Delta S$), such approach is not relevant for irreversible denaturation. Accordingly, thermodynamic description was avoided in the literature cited above and raw data were presented. We decided to study thermal denaturation of LTP1 in a broader temperature range (up to 120°C).

In our study, process of thermal denaturation of LTP1 covalently modified at Asp 7 was monitored by NMR and differential scanning calorimetry (DSC) up to 120°C. While DSC provided a complete picture of heat capacity changes, NMR selectively described the structural changes during protein unfolding.

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Determination of ammonium, calcium, magnesium, sodium and potassium in drinking water by capillary zone electrophoresis on a column-coupling chip

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Calcium, magnesium and sodium (major constituents) and ammonium and potassium (minor constituents) are one of the main indicators of drinking water quality. Therefore, there is a need for a continuous monitoring of their concentration levels in drinking waters. At present, ion chromatography is a dominant method used in routine analytical laboratories for the monitoring of these cations in waters. Nevertheless, capillary electrophoresis (CE) is a good alternative to IC with respect to the ionogenic character of the mentioned cations.

This work deals with the simultaneous determination of ammonium, calcium, magnesium, sodium and potassium in drinking water by capillary zone electrophoresis (CZE) on a column-coupling (CC) chip with suppressed electroosmotic and hydrodynamic flows. CZE separations were carried out in a propionate background electrolyte at low pH (pH = 3.2) containing triethylenetetramine as a surface modifier of the chip channels and 18-crown-6 at a 35 mmol/l concentration as a complexing agent to reach complete resolution of the cations. The concentration limits of detection obtained for the studied cations were ranged from 3.0 to 6.9 μg/l concentrations (corresponding to their 0.11 - 0.26 μmol/l concentrations) when a 900 nl volume of the sample was injected into the chip. A short total analysis time (ca. 300

s) and a dynamic range over two orders of magnitude (25-4000 µg/l) for the cations were reached using contact conductivity detection. Short- and long-term repeatabilities of the qualitative and quantitative parameters did not exceed 3.8 % of RSD for the peak areas and 2.6 % of RSD for the migration times of the analytes.

The analysis of five drinking water samples offered good repeatabilities of the migration times (RSD below 2.0 %) and peak areas (5.0 % of RSD or lower) of the analytes. Recoveries of the cations in drinking waters ranged from 93 to 106 %. Dilution and degassing of the drinking waters were the only sample pre-treatment procedures.

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Proteomic analysis of barley proteins by 1D-, 2D-HPLC and MALDI-MS methods

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Proteins determine the quality of barley in malting and brewing processes. In this regard, water-soluble barley proteins play a major role in the formation, stability, and texture of beer foams. Barley malt is usually the major raw material for the production of beer. There are several proteins that are resistant to the malting process and have been reported to be glycated **[1,2]**. Nonenzymatic protein glycation is caused by a Schiff's base reaction between the aldehyde groups of reducing sugars and the primary amines of proteins.

Here we present an application of 1D HPLC for determination of changes in the protein content after malting and 2D chromatographic separation of peptides after enzymatic digestion of barley extracts followed by MALDI-TOF/TOF analysis to identify proteins and determine nonenzymatic glycations.
The separation of proteins was carried out using monolithic column and separation of the peptides was performed with an on-line combination of two commercial, conventional HPLC columns. The first column was packed with a strong cation exchanger and the second one was C18 reverse phase column. The protein and peptide fractions were mixed with the matrix solution and spotted directly on a MALDI plate using ProBot.

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Quantitative determination of intracellular adenine nucleotides and coenzymes from bacterium *Paracoccus denitrificans* toward to targeted metabolome analysis

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Metabolomics (metabolome analysis) is comprehensive analysis of small molecules (metabolites) in the cell. Because of the large differences in abundance and wide diversity in physico-chemical properties of metabolites in organism the metabolome analysis is a difficult analytical task. So several approaches of metabolomics studies are known; e.g. the targeted metabolome analysis is focused only on quantitation of specific metabolites or metabolite classes.
The development of robust a consistent experimental protocol for all steps in the procedure ranging from biomass cultivation, metabolism quenching and metabolite extraction to the quantitative analysis is required.

Recently the selective and sensitive method for targeted metabolome analysis of adenine nucleotides (ATP, ADP, AMP) and coenzymes (NAD^+, NADH, $NADP^+$ and NADPH) based on field enhanced sample stacking-capillary zone electrophoresis combination has been developed. The main aim of this study was to optimize procedure for isolation and concentration of targeted metabolites from the biological matrix of bacterium *Paracoccus denitrificans* with the least possible losses.
Organic solvents like methanol, ethanol, acetonitrile and others are often used for extraction of intracellular metabolites from bacterial cells [1-3]. In this consequence these extraction media were tested: 20, 50, 80 and 100 % methanol, ethanol, acetonitrile and furthermore mixture of acetonitrile:methanol:water – 40:40:20, mixture of acidic (0.1 M formic acid-containing) organic solvents and mixtures of basic (0.1 M NH_4OH-containing) organic solvents. Metabolite yields of individual

extractions were measured by already optimized CZE method with UV detection and compared each other.

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On-line preconcentration of 4'-hydroxydiclofenac for MEKC separation by means of sweeping in homogenous electric field

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Cytochromes P450 (CYP) plays a key role in drug metabolism. Many analytical approaches to follow the drug metabolism by CYP were established. Micellar electrokinetic capillary chromatography (MEKC) is one of the most favorable method, particularly due to a low sample requirement and high efficiency, moreover CYP reaction mixture can be analyzed without any pre-treatment [1,2]. However, small sample volumes and short optical path-lengths lead to low concentration sensitivity of MEKC. In response to this problem, several on-line focusing methods have been developed to preconcentrate analytes inside the capillary before separation and detection.

In this study a method using sweeping in homogenous electric field for on-line preconcentration of 4'-hydroxydiclofenac was developed. Subsequently it was used for determination of CYP2C9 kinetic parameters. Main emphasis was put on substrate concentrations bellow 2 μM where a weak positive cooperation was estimated [1]. The 50 μm fused-silica capillary (56 cm effective length) was used to carry out all separations. 60 mM SDS in 20 mM phosphate 20 mM tetraborate buffer was used as a background electrolyte. Injection was accomplished by an application of 50 mbar pressure to the sample vial for 48 s. Separations were performed at 24 kV (positive polarity) and the temperature of capillary 25 °C. Analytes were detected using diode-array detector at 200 nm with a bandwidth 10 nm. Reaction mixtures containing 0,25-25 mM of diclofenac, 11,73 pM recombinant CYP2C9, 1 mM $NADP^+$, 6 mM glucose-6-phosphate, 35 $U \cdot ml^{-1}$ glucose-6-phosphate dehydrogenase, 5,1 mM $MgCl_2$,

0,1 M KCl and 50 mM phosphate buffer incubated for 30 minutes. Kinetic parameters were evaluated by SigmaPlot 8.02 software.

As the result Michaelis constant K_m = 4,62±0,38 μM, maximum reaction velocity V_{max} = 18,35±0,63 nmol·min^{-1}·nmol^{-1} and Hill coefficient n = 1,22±0,07 (p = 0,05; R^2 = 0,991) values were determined. Value of Hill coefficient confirms a presence of weak positive cooperativity in low substrate concentration region.

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Simple and fast approach for characterization of grain proteins and their modifications by combination of convective interaction media and MALDI-TOF MS

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Cereal proteins have important impacts on nutritional quality for humans and livestock and on functional properties in food processing. In the study, fast separation of technologically important grain proteins using short bed Convective Interaction Media (CIM®) disks with mass spectrometry (MS) identification is presented. Used monolithic disks present an ideal solution among the chromatographic columns due to their monolithic structure and extremely short length. They have very fast mass transfer between the mobile and stationary phase, which provides high speed and high efficiency of the separation.

Comparison of separation efficiency of CIM® DEAE and CIM® CM disks (BIA Separations, Ljubljana, Slovenia) for analysis of predominant proteins in barley malt and their posttranslational modifications (glycations) was investigated. CIM® DEAE disk represents a weak anion-exchange chromatography, while CIM® CM disk represents a weak cation-exchange chromatography. Both kinds of disks separate the malt extracts into three main chromatographic fractions, which were collected during the separation and purified using both ZipTipC18 and Nanosep 3K Omega treatment

devices prior to MS analysis. The collected fractions were analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. Our separation procedure using CIM® disks provides fast way of separation of modified proteins from malt (Lipid transfer protein 1, Lipid transfer protein 2 and fragment of Protein Z). These barley proteins survive the brewing process and they are very important for the formation and stability of beer foam.

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Preparation, physico-chemical properties and application in immunofluorescence analyses of CdTe quantum dots

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Quantum dots (QDs) are semiconductor nanocrystals in the size range from 1 to10 nm. Since QDs show unique optical properties as sharp and symmetric emission spectra, good chemical stability, practically no photobleaching and emission wavelength dependent on a nanoparticle size, they became suitable labels applied in fluorescence microscopy and analytical separation methods with laser-induced fluorescence LIF detection. QDs consist of semiconductor central core stabilized by charged compounds on the surface. The molecules attached to the outer shell determine particle hydrophilicity and serve as means for attaching ligands or conjugating biomolecules. The capability of the prepared quantum dots to be used as a selective fluorescent labeling has been tested.

We have prepared a set of water-soluble CdTe quantum dots by the chemical reaction of cadmium chloride and sodium hydrogen telluride at the presence of 3-mercaptopropionic acid (MPA) [1]. The fluorescence emission maxima were in the range from 500 to 750 nm. Thus, the particle sizes, determining the wavelength of the fluorescence emission, were evaluated to be in the range of 2 - 4 nm. The extraordinary broad excitation spectrum lies in the range from 300 to 550 nm with the absorbance maximum at 469 nm. The lifetimes of the fluorescence, determined by the time-resolved fluorescence spectrometry, vary from 3.05 to 20.5 ns with increasing size of the particles. The QDs size uniformity has been tested by slab-gel electrophoresis.

QDs can be conjugated with different molecules by several reactions. Commonly used succinyl imide (MPA) and carbodiimide (EDC) coupling agents mediate the

formation of peptide bond between a carboxylic group on the surface of QD and an amino group in a conjugated protein [2, 3]. We have conjugated MPA coated CdTe QDs with antiovalbumine using EDC nad NHS. The concentration of reaction products were checked by capillary electrophoresis with LIF detection.

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Comparison of electrophoretic methods for dextromethorphan metabolites separation prepared by *in vitro* incubations

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Characterization of phase I metabolites is an inevitable part of research and development of new drug entities. *In vitro* studies accomplished under the action of microsomes represent the appropriate tool for drug metabolism examination. Human liver microsomes credibly mimic natural enzymatic system involved in metabolism of different endogenous and exogenous compounds. Microsomes are the source of cytochromes P450, enzymes of phase I biotransformation. Dextromethorphan (DEX) was chosen as a probe substrate of cytochrome P450 (CYP) isoform 2D6. However, CYP isoform 3A4 also contributes to its metabolism three metabolites are expected; namely 3-methoxymorphinan, 3-hydroxymorphinan a dextrorphan. To study DEX transformation, off-line and on-line modes of incubation were tested.
Methods of capillary electrophoresis have been applied to different pharmaceutical and clinical problems, e.g. determination of drugs, metabolites and biomarkers [1-3]. The capillary is an implement of separation; on the other hand it can serve as a micro vessel as well.

The aim of this work is the comparison of methods applied on DEX metabolite separation. The metabolites prepared by off-line incubation were successfully separated in tetraborate buffer (80 mM; pH 9.85) at temperature 25°C. As for on-line mode both, incubation and separation conditions have to be compatible, the partial

filling method [4] and re-optimization of background electrolyte were necessary. The favorable metabolite separation was obtained with electrolyte containing linear polyacrylamide (10%, v/v) and 2-propanol (10%, v/v) at 37°C. The particular parts of methods, namely injection procedure, separation parameters and conditioning are discussed.

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Micro solid amalgam electrodes (SAE) array, fabrication and characterization

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Characterization of biological samples is demanding on analytical methods which need to be sensitive, selective, reproducible. Additionally, all the previous should be fulfilled when using very small amounts of the biological material. While fluorescence based techniques, especially with laser excitation, can fulfill all detection requirements, there are many cases when a simpler, less expensive, method is desirable. In the past it has been demonstrated that electrochemistry may provide sufficient sensitivity for biomolecule analysis. Desirable reduction of the required sample volume can be achieved by decreasing of the electrodes dimensions. Electrodes with micrometer area can also provide sufficient electrode density for parallel operation and further integration into microfluidic devices.

In contrast to solid electrodes, such as gold, platinum, carbon and indium tin oxide (widely used in various biosensors) mercury electrodes offer a different potential window, reaching highly negative potentials, not accessible by solid electrodes. With mercury electrodes reduction processes in nucleic acids and proteins, not accessible with the above solid electrodes, can be followed. Liquid mercury is, however, not

convenient for biosensors but application of SAE can solve the problem. To our knowledge no application of SAE in DNA and/or protein arrays has been reported. In this work we describe the preparation and characterization of simple micro SAE array for universal and/or selective biological specimen detection. The electrodes were prepared on a glass wafer substrate utilizing vacuum metal deposition and photolithography. The surface of the individual electrodes was separated by an insulating layer. Besides preparing electrodes of different dimensions the fabrication process also allowed testing of various metals. The electrochemical properties of electrodes were tested with respect to their size, material, stability and sensitivity using cyclic voltammetry. The microfabrication process showed excellent reproducibility allowing construction of simple disposable arrays.

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Pre-concentration and pre-separation of proteomic samples for MALDI-TOF/TOF mass spectrometry analysis using short monolithic columns

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Samples obtained in proteomics experiments are usually complex due to the presence of many peptides derived from enzymatic digestion of previously separated protein mixtures. The procedure that simultaneously allows biological sample clean-up, pre-concentration and pre-separation prior to mass spectrometry analysis with MALDI-TOF/TOF detection is presented.
In this approach, we have used a gradient elution for the pre-fractionation of peptides on short monolithic capillary columns. Monolithic columns were created inside the capillary via thermal-initiated, free-radical polymerization of ethylene glycol dimethacrylate and lauryl methacrylate monomers in the presence of 1-propanol and 1,4-butandiol as porogen system [1]. The model peptide mixture from tryptic digestion of iodoacetic acid alkylated bovine serum albumin was pre-fractionated employing the short monolithic column. The elution of peptides was achieved using a linear gradient of acetonitrile from 0 to 60% in water with 0.1% trifluoroacetic acid [2]. Individual fractions were collected on the MALDI target spots covered with alpha-cyano-4-hydroxycinnamic acid and these sample spots were analyzed using MALDI-TOF/TOF mass spectrometry. The obtained data were submitted into the Mascot database searching and compared with data without previous separation. The protocol was then applied for the analysis of real samples from barley protein extracts

separated using 1D SDS gel electrophoresis. The results show an improved quality of the analysis of peptide samples using the described approach.

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Application of polyakrylamide GE and MS for antifungal proteins in the barley and malt caryopsis

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Recently, a great attention has been paid to determination of various metabolites after pathogen-caryopsis interaction. Some of antifungal proteins, e.g. thionins (low molecular weight proteins with 3 – 4 disulfide bonds [1]) can be associated with beer quality – gushing [2]. The changes proceeding during pathogen-caryopsis interaction were monitored by the combination of Sodium Dodecyl Sulphate - Polyakrylamide Gel Electrophoresis (SDS-PAGE) and Matrix-Assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry (MALDI-TOF MS) with chemometric evaluation. The best extraction of thionins was achieved by 0.025 $mol.l^{-1}$ sulphuric acid after 4-hour shaking at room temperature. Separation of low molecular proteins in the barley and malt caryopsis was adapted according to Schager, von Jagow protocol [3]. Mini Protean 3 Cell device (Bio-Rad, Philadelphia, USA) was used. Composition of gel: 16%-10%-4% T, cross-linking 3% C. Separation was at 145 V for 60 min. The LOD was 10^{-6} $mol.l^{-1}$. Digestion [4, 5] was realized by 5-10 $mg.l^{-1}$ trypsin (Sequencing Grade Modified Trypsin, Promega, Austria) for 2 and 4 hours at 37 and 55°C, with and without prior reduction and alkylation. MALDI-TOF MS (Axima-CRF, Kratos Analytical, Manchester, UK) was applied for peptide fingerprint mapping. The amino acid sequences were ascertained from Expansy Proteomic server and theoretical protein cleavage, peptide mass fingerprinting data were observed through program ProteinProspector. The following matrices were used: 0.05 $mol.l^{-1}$ CHC (□-cyano-4-hydroxysinapic acid), and SA (sinapic acid), quick & dirty spotting of sample and matrix solution 1:1. Modifications of protein reflecting quality of barley and malt caryopsis were determined in dependence on variety and dissimilar grade of pathogen infection for 20 samples. All data were subjected to chemometric evaluation by program Statistica. Correlation of thionins' presence with their probable precursor was

observed just in barley. Interestingly, positive degree of beer gushing observed in malt caryopsis was found to correlate with thionins' occurrence in barley, not in malt.

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Diagnosing of inherited disorders of purine and pyrimidine metabolism from dry blood spots

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Disorders of purine and pyrimidine (P&P) metabolism are diagnosed by analysis of bases and riboside in extracellular fluid or intracellular nucleotides. Routine use of analysis of intracellular nucleotides is hampered by their instability. We report here a method for the analysis of intracellular nucleotides in dry blood spots.
Previously published method [Friedecky D et al. 2007, Eletrophoresis] is applied on separation of dry blood spots. Stability of dry blood spots within five days was tested and compared to immediately processed blood. Seven major nucleotides were quantified and statistically evaluated.
Nucleotides in dry blood spots are stable within five days at laboratory temperature. DBS compared to fresh blood offers higher ratio of GTP/GDP and NADP/NAD and lower ratios of ATP/ADP and ADP/AMP, respectively. Profile of all nucleotides are comparable and easy identifiable in electropherograms. Potential usefulness of the method was demonstrated on incorporation of the pathognomic ribosides into human erythrocytes. The ribosides were converted into ribotides. This model mimics state of inherited metabolic disorders.
Analysis of nucleotides in dry blood spots significantly contributes to better recognition of inherited disorders of nucleotide metabolism. Immediate processing of the sample and transport to laboratory are not necessary in case of DBS compare to fresh erythrocytes.

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Simultaneous determination of the waste products of nitrogen metabolisms and inorganic cations in human urine using CE with contactless conductivity detection

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Capillary electrophoresis (CE) with contactless conductivity detection (C^4D) was used to determine waste products of the nitrogen metabolism (ammonia and creatinine) and of biogenic inorganic cations in samples of human urine. Clinical laboratories routinely determine ammonia by enzymatic methods [1]; creatinine by using the Jaffé reaction [2]; K^+ and Na^+ ions are determined potentiometrically with ion-selective electrodes and atomic absorption spectrometry is used for determination of Ca^{2+} and Mg^{2+} ions [3]. A CE separation offers the possibility of simultaneous determination of all the ions in a single run, thus making the analysis considerably shorter.

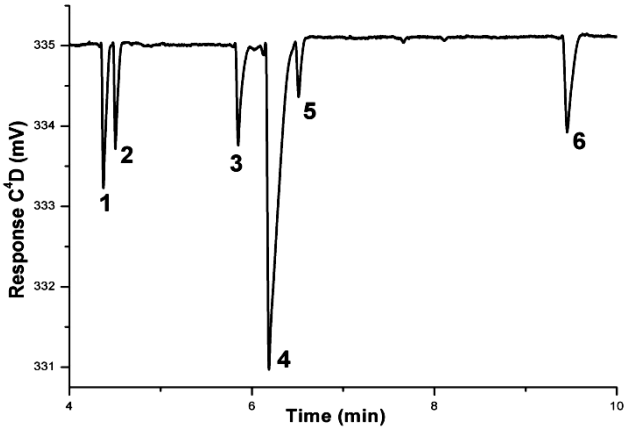

Figure 1. An electropherogram of 100times diluted human urine sample. Peak identification: 1 - NH_4^+ (480 mg.L^{-1}); 2 - K^+ (670 mg.L^{-1}); 3 - Ca^{2+} (280 mg.L^{-1}); 4 - Na^+ (2270 mg.L^{-1}); 5 - Mg^{2+} (78 mg.L^{-1}) and 6 - creatinine (980 mg.L^{-1}).
Capillary: 75 μm i.d., 80 cm total length, 64.5 cm to detector; Injection: 50 mbar for 9 s; Separation: +30 kV/+34 μA.

The complete separation of all the analytes in a model sample and in real urine samples was performed in the optimized background electrolyte consisting of 2 M acetic acid + 2 % (w/V) polyethylene glycol (PEG) [4]. The limit of detection values for the optimized procedure ranged from 0.8 μM for Ca^{2+} and Mg^{2+} to 2.9 μM for NH_4^+ (in terms of mass concentration units, from 17 μg.L^{-1} for Mg^{2+} to 102 μg.L^{-1} for creatinine). These values are adequate for determination of NH_4^+, creatinine, Na^+, K^+, Ca^{2+} and Mg^{2+} in real urine samples.

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Acknowledgements

This work was supported by the Grant Agency of the Czech Republic (grant no. 203/07/0896) and Grant Agency of the Academy of Sciences of the Czech Republic (grant no. IAA 400400704).

Comparative proteomic study of red yeast *Rhodotorula glutinis* cultivated under normal and stress condition

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Red yeasts are a wide group of aerobic yeasts that produce carotenoid pigments as a protection against letal effect of UV radiation of sunlight and oxidation damage. Carotenoids are lipofilic pigments that occur in photosynthetic plants (plants, bacteria) as well as non-photosynthetic organisms (bacteria, fungi). The composition of carotenoids depends strongly on the microorganism strain. One of the important producer of □-carotene, a precursor of vitamin A, is yeast *Rhodotorula glutinis*. It was shown recently that *Rhodotorula glutinis* produce larger amount of □-carotene under stress conditions. The objective of this work is the analysis of proteomic changes of stressed yeast, related to the increased production of carotene.

Yeast strain *Rhodotorula glutinis* CCY 20-2-26 has been cultivated under normal and stress conditions. The cells separated from the cultivation medium were lyophilised and the proteins were isolated using IPG buffer. Samples were analysed using 2D electrophoresis. Gel images from 2D electrophoresis were analysed using PDQuest analysis software and the differences between stressed and non-stressed samples were identified and quantified. Protein identification was performed after excission of the specific spot off the 2D gel and tryptic digestion. The resulting peptides were analysed by MALDI-TOF and LC-MS/MS techniques. The proteins were identified via database searching (National Center for Biotechnology Information).
Although the genome *Rhodotorula glutinis* has not yet been completely sequenced and the corresponding proteins are not included in databases, several proteins were identified usning a homology search against known organisms including Heat Shock Protein 90, Heat Shock Protein 70, GTPase or Protein Synthesis Elongation Factor 1-alpha.

Acknowledgements
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Determination of imatinib in plasma by capillary electrophoresis

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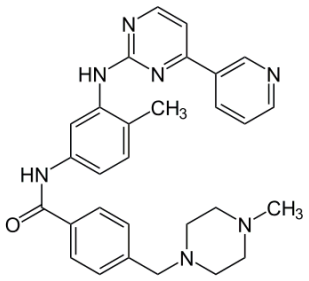

Gleevec (imatinib mesylate) is the first of a new class of antiproliferative agents called signal transduction inhibitors, which the pathways that signal the growth of tumor cells. The drug has been approved for oral administration in the treatment of chronic myeloid leukaemia in blast crisis.
Samples were prepared single in a simple and single step by precipitating the plasma proteins with methanol and supernatant was used for assay.
Final conditions were consisted of citrate buffer (60 mmol/l) adjusted with GABA (□-amino-n-butyric acid) to pH 4.0 with addition of methanol (10 %, v/v), electric field of 556 V/cm, 25 °C, hydrodynamic injection of 3 s, detection at 265 nm. Total analysis time was 8 min. Limit of detection is 80 nmol/L (S/N=3). In order to validation of the method linearity, imprecision, recovery and reproducibility of migration times were estimated. The method was applied on plasma samples from patients with chronic myeloid leukemia treated by Glivec.
Conclusion: We developed capillary electrophoretic method for determination of imatinib in plasma allowing analysis with sensitivity sufficient for clinical settings.

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Quenching of human cultured skin fibroblasts

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Metabolomic analysis of cultured cells requires effective removal of all potentially interfering substances from cultivation media with simultaneous metabolic quenching. The procedure needs to be rapid to avoid unnecessary metabolite losses due to cell lysis. We report here an optimized preparation method for metabolomic analysis of human cultured skin fibroblasts.

We used quenching solutions based on 60% methanol at – 40° C. Several manipulation techniques of quenching were tested with different volumes of quenching solvent. Quenching procedure was performed either with a pipette or a syringe with a needle. Manipulation techniques were optimized using saturated solution of amidoblack as a dilution marker. Leakage of intracellular metabolites during quenching procedure was assessed by the measurement of ATP by capillary electrophoresis and pulse-labeling with 14C-glycine and autoradiography in the quenching solution and cell extract.

Of all manipulation techniques tested, the best results were achieved by quenching the cells with 20 ml of solution with a syringe with a bent needle. Cultivation flask was held bottom-up and the solvent was sprayed-out. Another 2 ml of solution was added and cell layer was scraped for extraction. Calculated final volume of culture medium left in the cultivation flask was 2.7 nl, which contributes to intracellular volume by approximately 0.3%. This procedure takes just about 13 seconds. Cellular leakage is 3% for ATP and 4,5% for amino acids (derived from 14C-glycine).

We have developed a quenching method for cultured human skin fibroblasts. This method is rapid, causes minimal cell leakage and removes cultivation medium very efficiently. We consider this manipulation technique to be optimal for quenching of fibroblasts and possibly other adherent cell cultures.

Acknowledgment
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Coupled column capillary isotachophoresis/electrospray mass spectrometry for large volume sample analysis

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Modern instrumentation for capillary isotachophoresis with coupled columns provides an efficient means for rapid electrophoretic analysis of sample volumes of up to 30 microliters. Commercially available instruments are commonly equipped with conductivity and UV absorbance detectors. While these universal detectors perform well for quantitative analysis of known substances where a standard is available on-line coupling with mass spectrometry is highly desirable for trace analysis of unknown species. In this work we have tested a new coupled column electrophoresis system allowing direct electrospray interfacing with the ion trap mass spectrometer. Specifically, the effect of short capillary transfer lines, utilizing fused silica capillaries with different internal diameters, was tested with respect to optimum sensitivity and zone broadening. A sheathless electrospray interface was used and the ionization performance was monitored as a function of pressure conditions, electrospray voltage and sample flow rates. The system was applied for analysis of proteins, peptides and

metabolites in body fluids. During the analysis the first, wide bore preseparation capillary with 0.8 mm internal diameter served for removal of the bulk sample components and preseparation of the potentially interfering analytes. Final separation was then finished in the narrow bore capillary after the electronic column switching. Besides the unequivocal identification of the selected sample components the MS analysis has also increased the instrument sensitivity by more than 4 orders of magnitude when compared to the on-column conductivity detection.

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New types of protein-boronic acid conjugates, their characterization and use in analysis of glycated biomolecules

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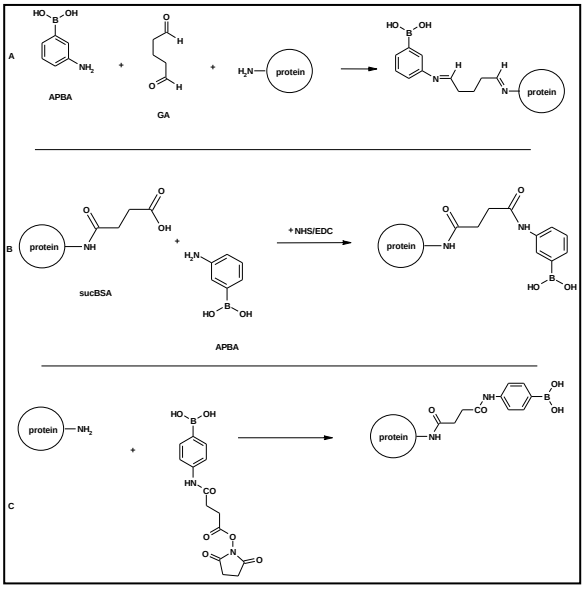
Boronic acid affinity to *cis*-1,2- and 1,3-diols is well known for more than six decades. Immobilized phenylboronic acids have been utilized to prepare chromatographic supports that selectively retain molecules having certain functionalities. Many important biological molecules including carbohydrates, ribonucleosides, and glycated proteins have been either analyzed or purified in this manner. Use of solid-phase immobilized boronic acid in separation of glycated hemoglobin fraction from non-glycated (followed by quantification) is probably the most significant application of affinity chromatography in a clinical praxis.



Modification of hydrophilic protein molecules (known as carrier proteins) with phenylboronic acid derivatives offers a possibility to perform affinity analysis of diol-containing molecules in a solution. Moreover, effective coating of gold nanoparticles and solid surfaces is possible in this way, too.

Various ways for protein-phenylboronic acid conjugates are described (see the scheme on the left). MALDI-TOF technique and TNBS colorimetric assay were used

for characterization of conjugate content. Affinity functionality of conjugates was in solution was successfully tested using fluorescent molecule containing diol-group (Alizarin Red S). Microtitre plates covered with these conjugates were used in analysis of glycoprotein (horseradish peroxidase), showing full functionality of the proposed concept.

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Nanopolyperoxidase – a universal system for simultaneous colorimetric signal enhancement and nanoscale visualization of antibody labeling

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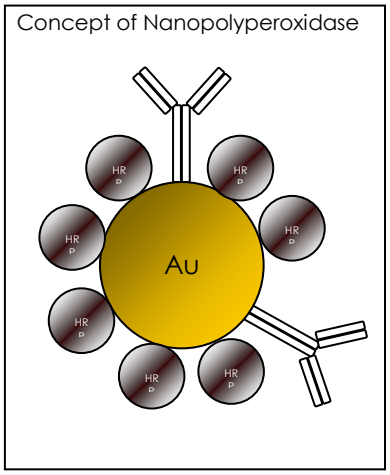
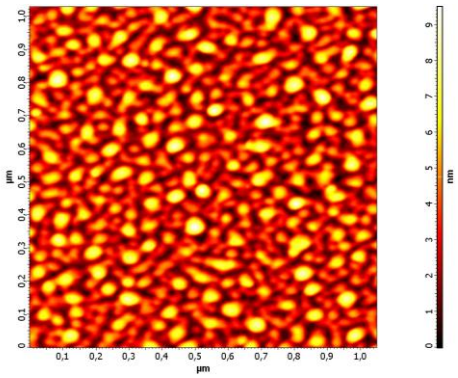


During past decades, a variety of signal amplification approaches in heterogeneous affinity immunoassays (including ELISA) has been developed. Those procedures are usually based either on recirculation enzymatic or repeated multiplication of an enzyme label via avidin-biotin system. From practical point of view, the multiplication procedure should not change experimental part of method, e.g. number of steps in procedure or solution content should not be changed.

For this reason, an idea of nanopolyperoxidase (NPP) has originated. The principle is simple: gold nanoparticles are modified with an antibody as well as with labeling enzyme (Horseradish peroxidase; ration 2:8). Modified nanoparticle is stabilized in a PEG solution.

Prepared NPP was studied using various methods. Atomic Force Microscopy (AFM) was used for characterization of structure and morphology in a nanometer scale (see image below). Use of NPP in construction of competitive ELISA as well as in



visualization of a protein (Human Serum Albumin as model compound) on a blotting membrane is presented.

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Chemically functionalized AFM tips: easy way to resolution enhancement in single biomolecule visualization

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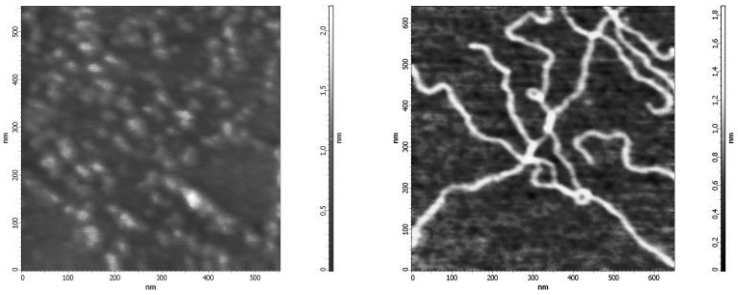
Atomic Force Microscopy (AFM) technique allows visualization of structures in a sub-nanometer scale, i.e. imaging of individual biomolecules. In principle AFM scanning probe, presented by ultra-sharp tip, is in close contact with surface and relief is visualized as 3D map (after conversion of a measured quantity, e.g. tip bending).

Resolution of an AFM microscope is essentially affected by the tip curvature (sharpness), however other experimental conditions would affect this parameter, too. Only weak and medium forces are usually employed in the interaction between tip and surface in the relief visualization. Surfaces visualized in a non-controlled atmosphere are naturally coated with a very thin (1-5 nm) layer of adsorbed water. In some cases capillary forces originating from this layer can strongly affect visualization process, as those forces are much stronger comparing to other intermolecular forces (van derWaals, hydrogen, ionic forces, etc.). Formation of capillary forces can be effectively suppressed by chemical modification of tip (mostly by hydrophobization of the tip) surface.

Various methods for tip surface modification are presented. Procedure always starts with the surface activation by a silanization. Subsequent coupling of either hydrophilic or hydrophobic molecules determines surface properties in this way.

Impact of the tip surface modification on resolution of images of individual biomolecules (proteins, nucleic acids) are shown as examples (on the right: IgG molecule-hydrophilic tip, DNA-lipophilic tip).



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Acknowledgment
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Preparation of protein conjugates with highly hydrophobic molecules and their use in a heterogeneous immunoassay

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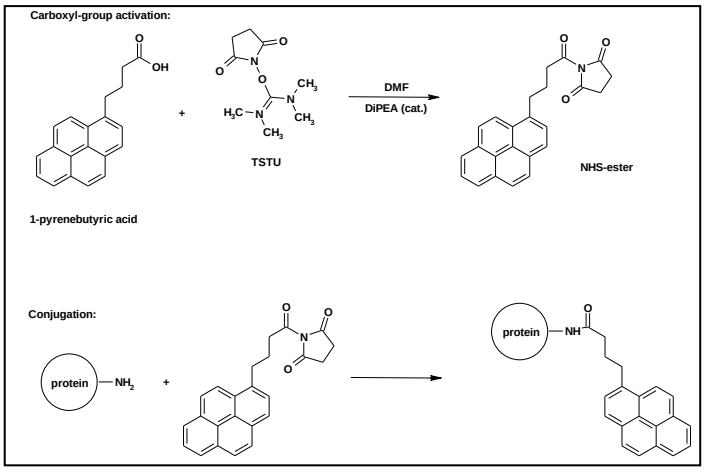

Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. PAHs occur in oil, coal, and tar deposits, and are produced as byproducts of fuel burning (whether fossil fuel or biomass). As a pollutant, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic.

Chromatogpraphic methods coupled to a mass spectroscopy are widely used in analysi of PAHs and their metabolites, as those methods provide high sensitivity and selectivity, combined with excellent robustness.

Immunoanalytical methods, such as ELISA, offer interesting possibility to detect PAHs at low levels with low expanses. Moreover, development of an antibody with specifity to polyaromatic hydrocarbons offers a possibility to detect DNA-PAH adducts.

Preparation and characterization of immunoconjugates of polyaromatic hydrocarbon model compound (pyrenebutyric acid) with proteins (carrier proteins and enzymes) is described (shown as scheme on this page). PAHs compounds as highly lipophilic molecules can not be conjugated to a protein structure in a single step procedure, however, step-by-step changing of conjugation and purification steps needs to be employed in this way. Use of prepared conjugates in construction of direct and indirect ELISA method is shown, too.

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This work was supported by The Ministry of Education, Youth and Sports of the CR, grant no. NPVII. 2B06056.

Off-line combination of preparative isotachophoresis with mass-spectrometry for analyses of biologically important analytes in complex matrices

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Busereline is synthetic analogue of natural gonadotropin releasing hormone (GnRH/LHRH). Busereline is important pharmaceutical substance and it is used for medical treatment of hormonal sensitive types of cancer, like the prostate cancer and the breast cancer. It is applied in very low doses (200 μg/day) and about 67% is excreted in urine in unchanged form during 6-24 hours. It means that busereline concentration in urine is very low.

Preparative capillary isotachophoresis can be used as very efficient sample pretreatment technique for the samples of biological origin. Fractions can be analyzed directly by ordinary separation technique (CE, HPLC, GC) or via the coupling of identification powerful detection system with powerful separation technique, e.g. CE-MS, HPLC-MS, GC-MS, GC-AED. Such off-line coupling is very simple especially in the case of coupling two compatible techniques using different separation principle.

In the present work we were studying the analytical possibilities of analysis of busereline when present in a complex (bio)matrix (urine) at a low concentration level by using off-line combination of preparative isotachophoresis – pITP with mass spectrometry (MS). MS becomes for some last decades extremely popular detection technique as it can provide both the qualitative as well as quantitative information for the many analytes present at very low concentration levels even in complex matrices.

MS analyses were carried out using electrospray ionization (ESI) in positive mode at 3 kV applied on spraying capillary and in the direct injection mode at a 2 ul/min constant flow rate. MS/MS spectra were obtained from $[M+2H]^{2+}$ ion using a 30 eV collision energy. A sequence of amino acids in busereline could be easily found due to the quality of obtained MS/MS spectrum. Even the concentration of busereline in urine samples were relative high (10 mg/l, reflecting at a 8.10^{-6} mol/l concentration), according to the ion intensities as obtained during MS and MS/MS analyses it is clear that the concentration of busereline in urine could be several orders of the magnitude lower. In fact, by using the pITP procedure before MS and MS/MS analyses, dramatic improvement of the analyte detection reliability by MS and MS/MS techniques was obtained.

Acknowledgments
This work was supported by grant of Slovak Research and Development Agency No. VVCE-0070-07, grant of Slovak Grant Agency No. 1/3558/06 and grant UK No. UK/206/2008.

Combination of electrophoretic and chromatographic techniques with mass spectrometry in analysis of biological samples

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Compounds of analytical interest, e.g. drugs and their metabolites, are usually present in biological samples (urine, serum, etc.) at (ultra)trace concentration levels. Therefore, their analyses represent the challenge for analytical chemists due to the complex character of biological samples consisting of several hundreds of different constituents having very wide span of chemical/biological properties and concentrations. Due to these facts, such analyses can be successfully performed when the powerful separation technique is combined with the sufficiently sensitive and/or selective detection method.

Gas and/or liquid chromatographic techniques are very often routinely used for such purposes. Techniques of capillary electrophoresis become more and more popular in last years due to their very favorite properties, like very high efficiency obtainable, very low consumption of the sample as well as the electrolytes used for the analysis. This feature is very important especially in the analysis of biological samples. Unfortunately, some techniques of capillary electrophoresis, mainly the mostly used zone electrophoresis, suffer from the detection sensitivity as in the most cases the signal of detector is connected with the I.D. of the separation capillary, which is very low. Mass spectrometric detection can solve this problem, as the signal does not depend on the capillary I.D. and it provides an excellent selectivity while keeping the sufficient detection sensitivity.

This work is dealing with different approaches to the analyses of short peptide (drug substance) present in urine at very low concentration level by using chromatographic and electrophoretic separation techniques coupled with mass spectrometry.

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