CECE 2007

4th international interdisciplinary meeting on bioanalysis

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Organized by:
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Introduction

Welcome to this year's CECE. Since its start we wanted to create an interdisciplinary meeting for informal communication of scientists from different sides of bioanalytical sciences. Starting with three lectures at the Institute of Analytical Chemistry in 2004 we have now outgrown the institute's conference room and expanded into a two day event with both lectures and poster presentations. The organizers want to thank you for your participation and hope that you will enjoy the scientific presentations, personal contacts and informal discussions.

2004

2005

2006

2007

Program

Thursday

9:00 – 9:15 opening

9:15 – 9:50
Acoustic manipulation of particles and cells for bioanalysis
Johan Nilsson

9:50 - 10:25
High sensitivity profiling and sequencing of complex carbohydrates by multi-capillary electrophoresis
Andras Guttman

10:25 – 11:00
Photopolymerised monoliths prepared using low uv light emitting diodes as a light source
Silvija Abele

11:00 – 11:35
Bionanotechnology at ANF data
Martin Polčík

11:35 – 12:10
Chiral nano-liquid chromatography-mass spectrometry applied to amino acids analysis for orange juice profiling
Salvatore Fanali

12:10 – 14:00 Lunch break – poster session

14:00 – 14:35
Chemoselective enrichment of tryptophan-containing peptides for applications in proteomics
Alexander Leitner

14:35 – 15:10
Portable LOC systems
Pavel Neužil

15:10 – 15:45
Porous polymer monoliths in microfluidic chips for separation of proteins and peptides
František Švec

19:00 Conference dinner

Friday

9:00 – 9:35
Major projects funded from structural funds in Brno
Stanislav Kozubek

9:35 – 10:10
Virus analysis by chip electrophoresis
Ernst Kenndler

10:10 – 10:45
Divergent flow isoelectric focusing
Karel Šlais

10:45 – 11:20
What are optical micro-manipulation techniques and what do they offer
Pavel Zemánek

11:20 – 11:55
***In silico* and *in vitro* protein engineering as a bioanalytical tool**
Jaroslav Koča

11:55 – 14:00 Lunch break – poster session

14:00 – 14:35
DNA banking and genetic information databases: New possibilities and same old fears.
Marek Minárik

14:35 – 15:10
Why not to use computers instead of CE instruments?
Bohuslav Gaš

15:10 - 15:45
Dušan Kaniansky

15:45 closing remarks

Abstracts

Lectures

Acoustic manipulation of particles and cells for bioanalysis

Johan Nilsson
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Acoustic standing waves will generate forces on particles in a fluid and can be used for positioning or trapping. Microfluidic systems are favorable to use since the forces are proportional to the system resonance frequencies that in turn are increased due to the shrinking dimensions. A second important aspect of microfluidic systems is the laminar flow conditions leading to predictable liquid flows that are crucial for a successful outcome. The presentation will discuss the fundamental aspects of acoustic manipulation in the MHz-range and show results from lipid separation from blood, post-surgery clean-up and size separation of particles. The latest findings from affinity selection of antibodies as well as the application of acoustic standing waves in a micro-FACS (Fluorescent Activated Cell Sorter) will also be discussed.
By generating a localized acoustic standing wave field, lateral forces can be used to trap particles or cell within the system. A 600 x 600 μm ultrasonic transducer integrated in the wall of a microfluidic channel is used for gentle trapping of particles and cells. Results from differential extraction of male and female DNA in forensics and an ATP release study from red blood cells will be demonstrated.

Biography

Johan Nilsson received his Master of Science in Electrical Engineering at Lund Institute of Technology in 1987. In 1993 he obtained his Ph.D. in Electrical Measurements on the topic Ink Jet and Droplet Technology at the Department of Electrical Measurements, Lund University. Following the Ph.D., he got a post-doc employment at the same department where he headed the research in droplet formation characterizations and silicon nozzle development. He developed the concept of flow-through microdispensing in 1995 and the topic for the research has since then been microfluidics and microstructures with a focus on microsystems for droplet based processes, protein analysis using mass spectrometry and acoustic manipulation for bio-analysis. He currently holds a position as Associate Professor and is since March 2000 the Head of the Department of Electrical Measurements at Lund University, Sweden.

High Sensitivity Profiling and Sequencing of Complex Carbohydrates by Multi-capillary Electrophoresis

András Guttman[1], Marcell Olajos[1], Heidelinde Glasner[1], Douglas Gjerde[2], Varouj Amirkhanian[3] and Ming Liu[3]
[1]Horváth Laboratory of Bioseparation Sciences, Innsbruck, Austria
[2]Phynexus, San Jose, United States
[3]eGene Inc., Irvine, United States

Capillary electrophoresis based fingerprinting and sequencing of complex carbohydrates is an important element of the recently growing systems biology endeavor. CE provides rapid and high efficiency separations and can be easily applied for profiling and sequencing appropriately labeled oligosaccharides. Application of a multi-capillary format significantly increases analysis throughput in revealing changes in the extent and/or nature of oligosaccharide distribution for the biotechnology and biopharmaceutical industry. As most sugars neither possess charged moieties nor have chromophores / fluorophores, their electrophoresis based analysis requires appropriate derivatization and sample preparation methods. 9-amiopyrene trisulfonic acid (APTS) is one of the most frequently used labeling reagents, supporting fluorescent detection in both laser and light

emitting diode (LED) induced formats. APTS also provides the necessary charges for electric field mediated separation. However, the very large molar excess requirement of the derivatization agent in the reaction mixture certainly represents a problem as the remaining un-conjugated reagent makes the analysis problematic in the region of < 5 GU. In this presentation we discuss various sample preparation options to remove the excess labeling reagent prior to capillary electrophoresis separation. Sephadex G10 filled multiscreen 96 well filter plate based method is compared to an automated robotic system using low volume pipette tips with Sephadex G10 and polyamide DPA-6S phase filled tips. All three methods enabled increased sensitivity analysis of complex carbohydrates but the DPA-6S phase filled tips worked particularly well enabling detection and evaluation of carbohydrate profiling and sequencing products. In sequencing applications, the high phosphate buffer concentration of the exoglycosidase digestion reaction mixture also caused difficulties with electrokinetic injection. Application of such volatile buffer systems as ammonium-acetate, ammonium-formiate and ammonium-carbonate prevailed in this problem.

Biography

András Guttman is holding a Marie Curie Chair Professorship of the European Commission and leads the recently formed Horváth Laboratory of Bioseparation Sciences (HLBS) at the University of Innsbruck in Austria. His main research interests are to pursue basic research in the field of bioseparation sciences with the aim to develop and implement high performance bioanalytical techniques for genomics, proteomics and glycomics based biomarker discovery. Dr. Guttman held prior appointments at Diversa Corporation (San Diego, CA) implementing bioindustrial scale carbohydrate analysis methods; at the Torrey Mesa Research Institute (La Jolla, CA) applying microfluidics methods to large scale genotyping; Genetic BioSystems (San Diego, CA), working on novel microgel electrophoresis platforms; and at Beckman Coulter (Fullerton, CA) developing capillary electrophoresis kits. In his postdoctoral work at the Barnett Institute (Boston, MA), he conducted basic research in the field of capillary gel electrophoresis. He has more than a 180 scientific publications, 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 Veszprem University (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.

Photopolymerised monoliths prepared using low UV light emitting diodes as a light source

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Institute of Analytical Chemistry AS CR, v.v.i., Brno, Czech Republic

Polymerisation initiated by UV light has been described extensively since the first synthesis of macroporous polymeric monoliths by photoinitiated polymerisation in 1997 by F.Švec and coworkers. The importance of photopolymerisation in separation science and its advantages in comparison to conventional way of polymerisation are widely described in the literature. Very successful synthesis of monoliths of various chemistries using different UV light sources (spectrolinkers, photochemical reactors, UV tubes or UV lamps) has been reported over the last decade.

In the presented work photopolymerisation has been conducted using a single low-cost UV light emitting diode (LED) as a light source in contrast to the classical bulky and costly irradiation devices. In comparison to other UV sources LED offer several advantages – low costs, long life time, small size, robustness, small heat generation and high light intensities. Polymethacrylic monoliths have been synthesised in transparent fused silica capillaries or in the channels of microfluidic chips using 2,2-dimethoxy-2-phenylacetophenone (DAP) or Michler's ketone (MK) as photoinitiators. The effect of LED optical power, polymerisation time, distance and position of LED from the capillary are investigated. Obtained monoliths are characterised by scanning electron microscopy (SEM).Very interesting effect of matching the wavelength of absorbance maximum of initiator with the emission wavelength of LED is presented.
Another example describes the use of UV LED for evanescent wave photopolymerisation. Photopolymerization by evanescent waves has been previously suggested for fabrication of very thin polymer films. Capillary used for photopolymerisations can serve as an optical wave guide what can result in formation of evanescent field at the inner surface of capillary. In

such a way photopolymerisation is started at the walls of capillary moving slowly to the bulk of polymerisation solution in the centre of capillary. By controlling the irradiation time monolith only around the walls can be obtained resulting in open tubular capillary column which could be used for liquid chromatography, capillary electrochromatography and related separation methods. Surface polymerisation using evanescent field could help to overcome common problems in preparation of monolithic columns, such as uneven polymer quality or formation of voids along the polymer bed and difficulties to control the thickness of polymer created on the surface.

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Biography

Silvija Abele graduated from University of Latvia in 1994. The same year she started her PhD thesis which was done in collaboration between University of Latvia and University of Claude Bernard Lyon I in France under supervision of Prof. ZICMANIS in Riga and Prof. GUYOT in Lyon. She has defended her thesis *"Preparation and Characterization of Reactive Surfactants Derivatives of Maleic Anhydride, Utilization in Emulsion Polymerization"* and got her PhD in Chemistry of Materials in 1998.
In 1999 Dr. ABELE started her postdoctoral fellowship in CNRS/ATOFINA research laboratory in Paris under supervision of Dr. Ludwik LEIBLER. The project *"Structuration of reaction medium by sequenced or grafted copolymers"* was focused on dispersion copolymerization in aqueous medium, polymer melt processing and mechanical testing and dynamic mechanical analysis of obtained polymers. After her return to the University of Latvia

in 2001 she continued in research and chemistry teaching for 3.5 years. In 2006 Dr. ABELE joined the group of Microfluidic Separations in School of Chemical Sciences of Dublin City University where she is a leading member of the research group of Dr. Mirek MACKA. Main field of her present research is synthesis of methacrylate monoliths using photopolymerisation and light emitting diodes as a light source. The aim of the project is to create monolithic phases in the channels of microfluidic chips for sample preconcentration and use as electroosmotic pumps in lab-on-chip devices.

Bionanotechnology at ANF DATA

Martin Polcik
ANF DATA (Siemens), Brno, Czech Republic

ANF DATA (Siemens IT Solutions) has started several projects in life sciences in order to secure future benefits which are expected to be significant in this field in the future. The projects deal with bioinformatics, i.e. the application of computers in biology, and nanotechnology which is closely related to the former via on-chip integration of different devices such as biosensors, micro fuel cells etc.

In this contribution the bionanotechnology activity in the field of liquid atomic force microscopy (AFM) will be presented. It is a result of a collaboration between Siemens and the group of Prof. S. Jarvis from the Trinity College Dublin, Ireland that targeted biologically relevant applications of AFM. AFM is a powerful method that provides quantitative information about systems such as cells, membranes, DNA etc. in their natural environments – liquids (such as water or buffer solutions) and which can achieve molecular or even atomic resolution. A special method based on the thermal vibrations of the AFM cantilever to calibrate its sensitivity and stiffness has been developed. It enables the user to perform fast and non-contact calibration of the two parameters - this is important especially when functionalized tips are used for which the standard contact method cannot be applied. An example of a functionalized AFM tip is shown in Fig. 1, where a multi-wall carbon nanotube has been used to functionalize the tip.

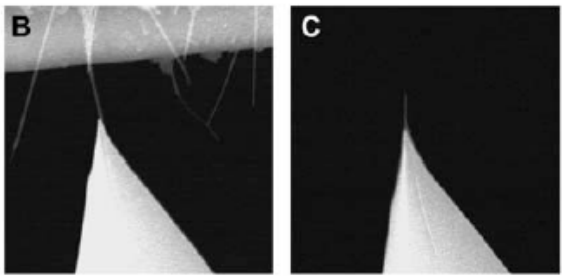

Fig. 1 Carbon nanotube functionalized AFM tip (M.Higgins et al., Biophys. J. **91** 2532 (2006))

The measurement using the functionalized tips have been carried out using the frequency modulation technique which provides quantitative information on the measured forces between the tip and the surface for arbitrary amplitude of the cantilever resonance vibrations (J. Sader et al., Appl. Phys. Lett. 84 1801 (2004)).

The carbon nanotube functionalized tips and the frequency modulation technique have been used to study the interface between lipid membranes and water; the solvation structures have been investigated and quantitatively analyzed. The results as well as some possible applications will be presented.

In the presentation, further Siemens PSE live science projects will be briefly introduced:

- RNA Workbench – prediction of RNA interference by which genes can be silenced including some interesting new results.
- Laboratory management for proteomics – system for automated analysis of proteomic data from mass spectrometry

Biography

Martin Polcik

Author of more than 70 publications in the area of Solid state physics, Surface physics and recently biology.

Occupation

September 2004 – Present

- Senior researcher, ANF Data (Siemens) Brno, Czech Republic

April 1998 – September 2004

- Visiting scientist, Fritz-Haber-Institut der MPG, Berlin, Germany

March 1995 – March 1998

- Postdoctoral research assistant, Fritz-Haber-Institut der MPG, Berlin, Germany

October 1991-February 1995

- Research associate, Laboratory of X-ray spectroscopy, Institute of Physics, Prague, Czech Republic

1991

- CSc. (PhD) degree from the Institute of Physics of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia

Chiral nano-liquid chromatography-mass spectrometry applied to amino acids analysis for orange juice profiling

Salvatore Fanali[1], Zeineb Aturki[1], Giovanni D'Orazio[1], Anna Rocco[1], Alejandro Cifuentes[2]

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[2]Department of Food Analysis, Institute of Industrial Fermentation (CSIC), Madrid, Spain.

Accurate analytical systems are required in order to determine safety, authenticity, processing, or contamination of food. Nano-liquid chromatography (nano-LC) is useful to that aim and offers several advantages over traditional methods. Among them we can mention: reduced consumption of mobile phase and sample, small amount of stationary phase, low toxic waste, short analysis time. Additionally the reduced flow rate allows good coupling with MS. Determination of amino acid enantiomers can be employed for quality control, contamination detection, processing monitoring of food products, etc. In fact the presence of D-isomers may indicate for instance adulteration or microbiological contamination of foods.

In this work the content of amino acid enantiomers in orange juice was assessed. Amino acids, after derivatization with fluorescein isothiocyanate (FITC), were focused on a C18 cartridge and then separated in a capillary column (75 □m i.d.) packed with vancomycin modified silica-diol particles.

The effect of some experimental parameters, such as pH and buffer concentration on enantioresolution and retention factor, was studied for optimizing the separation of D- and L- FITC-amino acids. The

chromatographic separation system was coupled with an ion trap mass spectrometer through a nano-spray interface in order to identify the analytes. LOD values as low as 8 ng/mL were reached for all the investigated samples. The method was applied to the comparative analysis of two different orange juice samples. Fresh natural vs. commercial juice were tested confirming their quality. The chromatographic profiles revealed the absence of D-isomers confirming the good quality of the juices. Lower concentration of L-aspartic acid was monitored in the fresh juice probably due to the origin and season of the collected fruits

Chemoselective Enrichment of Tryptophan-containing Peptides for Applications in Proteomics

Leitner A., Föttinger A., Melmer, M., Lindner W.

Department of Analytical Chemistry and Food Chemistry, University of Vienna, Vienna, Austria

The enormous complexity of biological samples still overwhelms the capacity of modern analytical workflows in proteomics. Therefore, the simplification of complex peptide mixtures such as those resulting from the enzymatic digestion of serum or cell lysates is an important sample preparation step prior to mass spectrometry-based protein identification. To this end, different strategies have been employed, such as multidimensional chromatographic or electrophoretic techniques that make use of general physicochemical properties of proteins and/or peptides (such as hydrophobicity, charge, molecular mass, etc.). Alternatively, enrichment techniques that allow the selective recognition of functional groups in the analytes of interest have been used for some time. This includes methods for the enrichment of post-translationally modified peptides, but also for those peptides carrying certain (rare) amino acid residues, e.g. cysteine.

Tryptophan (Trp) is another rare amino acid, although specific enrichment techniques for Trp-containing peptides have not been widely used in proteomics up to now. In previous work with malondialdehyde (MDA) as a modifying reagent (1), we observed a side-reaction with Trp residues that

had not been reported before. In fact, MDA reacts with the nitrogen of the indole group, forming an α,β-unsaturated, acrolein-type aldehyde moiety that is available for further reactions, for example with hydrazines or hydrazides (2). Based on this MDA chemistry, we have developed a novel enrichment scheme to isolate tryptophan-containing peptides from complex mixtures (3). We report on the development of this methodology and its successful application to yeast cell lysate as a model biological sample, using nanoflow HPLC and tandem mass spectrometry. The results demonstrate that this chemoselective procedure allows a more comprehensive profiling of protein mixtures by reducing the complexity of digests.

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(3) Foettinger, A., Leitner, A., Lindner, W. *J. Proteome Res.* 2007, *6*, 3827-3834.

Biography

Alexander Leitner studied Chemistry at the University of Vienna where he graduated in 2001. He obtained his Ph.D. at the Department of Analytical Chemistry, University of Vienna, in 2004, working on the development of chemoselective recognition principles and their application to mass spectrometry under the supervision of Prof. Wolfgang Lindner. Since then, he continues his research in the Lindner group as an Assistant Professor.
His main research interests are the use of mass spectrometry and liquid chromatography-mass spectrometry for the analysis of biomolecules. This includes the development of chemoselective reaction and recognition principles for analyzing peptides, proteins and their (post-translational) modifications. He is involved in a number of interdisciplinary projects, including the "Austrian Proteomics Platform", a network of Austrian academic research groups related to proteome research, and a cooperation with the Medical University of Vienna, studying the role of oxidized phospholipids in inflammation processes.

Portable LOC systems

Pavel Neuzil
Institute of Microelectronics, Singapore

Compact autonomous and modular LOC system for diagnostics will be presented. Depending on selected modules it can performed sample preparation, real-time (RT) PCR, melting curve analysis, capillary electrophoresis, localized surface plasmon resonance (LSPR), electro - chemical sensing or label-free sensing based on nanowires. The signals originated from the sensing part are processed by miniaturized four channel lock-in amplifier. Its outputs are digitized and further processed by a single chip controller. Four inch liquid crystal touch-screen display is used to control the system and display collected data. The whole system is enclosed in an aluminum casing and has a diameter of 100 mm, a height of 60 mm, and a weight of only 150 g. Results from label free nanowire sensor, LSPR and real-time PCR will be shown.

Biography

Pavel Neuzil received his M.S and Ph.D. degrees from the Faculty of Electrical Engineering at Czech Technical University in Prague. Since then, he has worked in different areas of electrical engineering such as CMOS design and manufacturing, as well as the multidisciplinary field of chemical and physical sensors. Currently, he is involved in a development of portable Lab-on-a-Chip systems for diagnostics.

Porous Polymer Monoliths in Microfluidic Chips for Separation of Proteins and Peptides

Frantisek Svec
The Molecular Foundry, E.O. Lawrence Berkeley National Laboratory, Berkeley, CA 94720-8197, USA.

Separations will continue to be an important part of all complex analytical systems no matter what size they may have. Therefore, incorporation of separation schemes in the microfluidic devices is so important. Obviously,

the simplest implementation includes separations in electrophoretic mode. However, electrophoresis is not a universal method and additional modes enabled by specific interactions of separated compounds with well designed surface chemistries are also needed. Packing microchannels with particulate is very difficult. In contrast, in situ preparation of porous monoliths requires only liquid precursors that facilitate filling of the channels. The polymerization process is then initiated by UV light or heat. Composition of the polymerization mixture enables good control of both porous properties of the monolith and its surface chemistry. Once located in the channel, the monolith can be used for separations in HPLC mode using pressurized flow or in electrochromatographic mode employing electroosmotic flow. Examples of the preparation and use of monoliths in such channels will be discussed in detail.

Biography

Frantisek Svec received both degrees B.S. in chemistry and Ph.D. degree in polymer chemistry from the Institute of Chemical Technology, Prague (Czech Republic) in 1965 and 1969, respectively. In 1976 he joined the Institute of Macromolecular Chemistry of the Czechoslovak Academy of Sciences where he was promoted through the ranks to the Head of Department and the Scientific Secretary of the Institute. He accepted an offer and joined faculty at Cornell University in 1992. Since 1997, he is appointed at the University of California, Berkeley. He is also visiting professor of analytical chemistry at the University of Innsbruck, Austria, and currently works as Lead Scientist in the Lawrence Berkeley National Laboratory. Dr. Svec is the author or co-author of over 340 scientific publications, he edited 2 books, and authored 75 patents. He is Editor-in-Chief of the Journal of Separation Science and member of editorial boards of a number of renowned journals including Journal of Chromatography A, Electrophoresis, Applied Macromolecular Chemistry and Engineering, and Chinese Journal of Chromatography. In 2003 he was elected President of CASSS (formerly California Separation Science Society). In 2005 he was awarded with M.J.E. Golay Medal in Chromatography and EAS Award for Achievements in Separation Science. In 2006, he obtained in Sweden a Honorary doctorate of philosophy and he has been selected as the 2008

recipient of the ACS Award in Chromatography. F. Svec is best known for his research in the area of macroporous polymers in different shapes such as beads, flat sheets, and, in particular, monoliths. His studies involve use of these materials in numerous applications including liquid chromatography, electrochromatography, supports for solid phase chemistry, enzyme immobilization, and microfluidics.

Major Projects Funded from Structural Funds in Brno

Stanislav Kozubek, Institute of Biophysics ASCR, v.v.i., Brno

According to the Operational Programme „Research and Development for Innovations" Centers of Exellence will be supported as Major projects.
In the frame of this Operational Programme 2 projects have been proposed from the Brno region - Central European Institute of Technology a Czech National Synchrotron Laboratory. Both projects represent large investments into research infrastructure, where integration into European Research Area is supposed as well as practical outputs and education of new scientists in the region. Obviously, existing human potential is of critical importance. The projects will be characterized and their interrelation will be discussed.

Virus Analysis by Chip Electrophoresis

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Electrophoretic experiments on microchip devices have been mostly directed to analyses of viral RNA or DNA fragments, but analyses of intact virions have to date not been presented. Exploiting the advantages of miniaturization of analytical devices we explored the conditions for the analysis of viruses, subviral particles, and virus-receptor complexes on microfluidic chips. To allow for detection via laser induced fluorescence the viral capsids were fluorescent labelled. This allowed to analyze Human

Rhinovirus serotype 2 and subviral particles, to follow the complexation of the virus with a synthetic fragment of the VLDL-receptor, and to track the heat-induced conversion of intact virions into empty capsids. Analyses were accomplished within few tens of seconds. In contrast to fused silica capillaries, the glass micro channels allowed for electrophoresis of the analytes without detergent, a prerequisite for the investigation of virus-liposome complexes.

Biography

Ernst Kenndler is Professor for Analytical Chemistry at the University of Vienna, Austria, and Docent at the University of Helsinki, Finland. From 1986 to 1999 he was lecturer at the Academy of Fine Arts, Vienna. His research areas include the theory and application of separation methods, with special emphasis on electrophoresis in the capillary format and chromatography. He is author of about 200 scientific publications. His main work is directed to bioanalysis, especially in the field of macromolecular assemblies like viruses. He is further working on theoretical aspects of capillary electrophoresis with organic solvents and their practical implications for separation.

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 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. To obtain reasonable sample throughput and zone capacity, high voltage drop over reasonable channel width has to be applied. Nevertheless, CF IEF has been examined also in micro-fluidic channels. Fast separations are expected while sacrifying separation efficiency and loadability.

In all CF IEF devices used till now the rectangular separation space with parallel flow and constant perpendicular potential over the channel length has been used. It means that input solution is subjected to the same total transversal voltage drop as the solution at the channel output. However, it is well known that in IEF with carriers the conductivity of the separation medium decreases by up to two orders of magnitude during the focusing run. Thus, in IEF, the voltage has to be increased during focusing to maintain stable wattage fed to area unit of the separation channel.

The solution some of above compromises of continuous flow IEF is seen in combination of the features of small channels with those of large ones. The basic idea is the continuous widening of the flat channel while the liquid flows from channel inputs toward the outputs which generates a divergent flow and, at the same time, to use small voltage at the channel input and high voltage at the channel output. The features of divergent flow isoelectric focusing (DF IEF) are demonstrated on simple devices using colored synthetic ampholytes. The final issue of the suggested method is that divergent flow isoelectric focusing enables faster focusing and/or higher performance in comparison with CF IEF in channels of constant width.

Biography

Karel Šlais received his MS degree in organic chemistry at Masaryk University, Brno in 1973, his PhD degree received in analytical chemistry at Institute of Analytical Chemistry, Brno headed by Prof. J. Janák at 1979. At that time his main scientific interests were micro column and open tubular liquid chromatography, enrichment techniques and electrochemical detection, his private hobby was electronics. This topic studied also at his stays under Prof. J. F. K. Huber at University of Vienna in1980 and under Prof. R.W. Frei at Free University of Amsterdam in 1986. After receiving the DrSc. degree in analytical chemistry 1992 he took the position of head of Department of Liquid Phase Separations at Institute of Analytical Chemistry, AV ČR, Brno. Recently, his main interests include isoelectric focusing and synthetic standards of isoelectric point. He is author and co-author of more than 90 scientific papers, supervisor of PhD students, and referee in several analytical journals.
More details see: http://www.iach.cz/departments/lps/www/

What are optical micro-manipulation techniques and what do they offer

P. Zemánek
Institute of Scientific Instruments ASCR, v.v.i., Brno, Czech Republic

The paper will briefly present the principles of optical micromanipulations and their latest development like holographic optical tweezers, Raman optical tweezers, and also optical traps integrated with micro-fluidic channels. Selected applications will be highlighted for example optical sorting of microobjects according to their sizes or properties, long-range objects delivery, measurement of pN interactions between micro- and nanoobjects, laser induced fusion of selected liposomes with different contents, optically controlled micro-pumps and micro-valves.

Biography

Pavel Zemánek received M.Sc. in physics at Faculty of Science of Masaryk University, in 1994 he got Ph.D. in plasma physics from Faculty of Science of Masaryk University but one year of this study spent at Clarendon Laboratory, University of Oxford as a Soros scholar on laser cooling and trapping of atoms.
Since 1991 he was the employee of the Institute of Scientific Instruments of ASCR, serving as the head of the Group of optical micromanipulation techniques (since 1994), head of the department of Coherence optics (2000-2003), member of the Scientific Council at ISI (since 1997) and deputy director (since 2001).
He dealt with (in historical order) frequency stabilizations of lasers, laser physics, laser spectroscopy, atom cooling and trapping, optical tweezers, optical scalpel, laser induced polymerization of micro-objects, optical sorting, delivery and self-arrangement of micro-objects and sub-micrometer size objects.
Since 1995 he was the principal or joint investigator of 10 projects supported by various Czech providers and one EU FP6 – ATOM3D.
He was awarded by Josef Hlávka price for young scientist by "Nadání Josefa, Marie a Zdeňky Hlávkových" (1998) and Otto Wichterle Award for promising scientist from Academy of Sciences of the Czech Republic (2003).

In silico and *in vitro* protein engineering as a bioanalytical tool

Jaroslav Koča
National Centre for Biomolecular Research, Masaryk University, Brno
jkoca@chemi.muni.cz

Protein engineering is a procedure of developing useful or valuable proteins by mutations of residues. It is usually performed *in vitro*, when the original DNA is mutated, incorporated into host cells (usually *E. coli*) and the resulting protein with mutated residues is expressed. It can also be performed *in silico*, when mutations are performed on a model in a computer.

Our effort is to connect both approaches to increase efficiency of the procedure. In our case, the engineered molecules are called lectins, nonenzymatic proteins that recognize carbohydrates. The lecture will be mainly focused on *in silico* part of the procedure, which is performed by the program TRITON developed in our laboratory. During this procedure, mutants are automatically generated, refined and their affinity and specificity is predicted. The method is then complemented by *in vitro* approaches that finish with thermodynamic and kinetic measurements.

Biography

Jaroslav Koča, Prof, D.Sc. is the director and a group leader in the National Centre for Biomolecular Research, Fac Sci, Masaryk University. He received his PhD (1979) in chemistry from Komensky University, Bratislava, Slovakia, and D.Sc. in organic chemistry (1993). He spent several years abroad as an NTNF postdoctoral fellow (2 years, Trondheim, Norway), visiting professor at Pacific Northwest National Laboratory (1 year, USA), in Rennes, Nantes, and Grenoble (France), Athens (Greece). His current scientific interests are focused on structural bioinformatics, computer simulations and computational chemistry of key biomacromolecules and their complexes. He has published more that 110 papers in competitive scientific journals including J. Am. Chem. Soc., J. Biol. Chem., Biophys J., Biochemistry, Structure, Bioinformatics, TIBS, J. Mol. Biol., Nucleic Acid Res. He has been involved also in managing science. He has been a coordinator of several large research projects.

DNA banking and genetic information databases: New possibilities and same old fears.

Marek Minarik, Laboratory for molecular genetics and oncology, Genomac International, Ltd., Prague, Czech Republic

Introduction of new generation of DNA sequencing platforms has a potential to shape genomic research in many ways. The significant reduction of cost and high speed of sequence readout allows to decipher genetic code of many organisms as well as to study minute variations among large cohorts of individuals. The recent revelations of the non-anonymous complete sequences of James Watson and Craig Venter as well as an announcement of "Personal Genome" project are the first steps towards personalization of the complex genetic information.

The sudden availability of such information is prompting initiation of association studies using large sets of DNA samples in order to decode complex genotype-phenotype associations. It is expected that such studies will undoubtedly promote creation of new types of multipurpose genetic databases. Whether such information will ultimately be utilized in clinical care or in identification and security sectors, it is already now a subject to scrutiny due to inherent potential privacy issues.

Biography

Marek Minarik has finished undergraduate studies in physical chemistry in 1994 in Bob Gas's group of electromigration processes at the Charles University in Prague. After completing 1 year of research work with Ernst Kenndler at the Institute of Analytical Chemistry in Vienna, he moved to USA to get his Ph.D. in bioanalytical chemistry working in Barry Karger's group at the Barnett Institute, Northeastern University in Boston. His primary focus was on development of instrumentation and applications for CE-based separations of DNA and proteins with main interest in micropreparative fraction collection. After receiving his Ph.D. in 2000 he assumed position in R&D application development at Molecular Dynamics (later acquired by Amersham Pharmacia and GE Healthcare) in Sunnyvale California. In 2002, he returned to Prague to start his own biotech company Genomac International. Today, Genomac is the largest private genomic

research center as well as provider of genetic testing in the Czech Republic with 13 employees, 4 doctoral and 2 diploma students. Dr. Minarik is author of more than 30 scientific papers including 2 issued US patents and numerous patent applications.

Why not to use computers instead of CE instruments?

Bohuslav Gaš
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Basic laws describing transport of charged compounds in electrolyte solutions can be formulated relatively easily. Here belong the continuity equation, acid-base equilibrium equations, interaction equations, and Navier-Stokes equations are employed. The question is whether these equations can be solved to get the picture about the phenomena taking place during electromigration. At present, the exact analytical solution can be obtained only in simplified configurations. For information about the most practical cases other approaches must be used, such as linearization of the equations, or their numerical solution. The computers available in all labs together with powerful software enable to implement all the mathematical tools to get deep information about behavior of the electrophoresis systems Then the composition of the electrolytes used and instrument configuration can be optimized to reach better separation without a necessity to use "wet chemistry". This can spare considerable time for method development and decrease consumption of chemicals.

The lecture gives an overview of software tools available for such purpose and demonstrates their performance.

Biography

Bohuslav Gaš is a professor of physical chemistry and leader of the group of electromigration separation processes at the Faculty of Science, Charles University, Prague. His present research interests include theory of

transport processes in solutions and methodology and instrumentation in capillary and chip electrophoresis.

TBA

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Posters

Regular properties of simple electrophoretic BGEs with multiprotic weak acids: Discovery of complex hybrid system boundaries

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In electrophoresis, the introduction of a sample zone into the BGE creates two new zone boundaries, the front and rear boundary of the sample zone which is sandwiched between the BGE. During an electrophoresis run, these boundaries move and split into another new boundaries demarcating the zones of analytes. Besides the analyte zones migrating out of the original sample location, system zones may be also formed. Such zones are formed by the BGE components and differ from the BGE only in their concentrations. The front and rear boundaries of such system zones evolve during electromigration and may show sharp (S), dispersed (D) and/or hybrid character. This contribution brings the results of theoretical and experimental investigation of system properties of very simple BGEs formed by one weak acid and one weak or strong base provided that the acid is polyprotic. It is shown that system boundaries of the hybrid type occur even in these simple systems represented, e.g., by the very common phosphate buffer. Theory reveals that a phosphate buffer system (formed by phosphoric acid and a strong cation) may exhibit very unusual complex nonlinearity of the EMD velocity curve showing three turns. Such nonlinearity leads to the formation of complex hybrid boundaries having D-S-D, S-D-S, or even S-D-S-D or D-S-D-S concentration profile types that are reported here for the first time. The velocity diagram method allows theoretical analysis of such complicated profiles and the results are in good accordance with both computer simulation and experiments.

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Rigorous Statistical Analysis of Mobility Curves for the Determination of pK_A of Weak Acids

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This contribution brings a new rigorous and complete statistical approach to the data processing of the mobility curves of univalent weak acids. It is based on fundamental equations for the related mobility curves, on applying the mathematical principle of the best fit, derivation of the related nonlinear equation and finding the mathematical solution of it which gives the best estimates of the mobility curves parameters. The mathematical procedure presented here does not impose any limitations upon the numerical data used, i.e., the experimental values of mobilities and pH may be real numbers (positive, negative, zero). Further, rigorous explicit statistical formulas are derived for standard deviations of effective mobility, dissociation constant and ionic mobility of fully dissociated acid in question.

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On-line Preconcentration via Electrokinetic Accumulation in Capillary Electrophoresis

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Capillary electrophoresis (CE), using common UV detectors, usually suffers from a lack of detection sensitivity due to small optical path. Necessity of analyzing low concentrations of bioactive compounds led to the establishing preconcentration methods prior the separation. Moreover CE provides interesting possibilities by using on-line preconcentration methods like stacking, sweeping, isotachophoresis or dynamic pH junction methods. These techniques do not need off-line operations and therefore they are easily handled.
One of the on-line preconcentration methods was developed in our laboratory [1]; it is based on electrokinetic injection of the sample in high pH electrolyte to the pH boundary formed by electrolyte with low pH (accumulation part). After a certain time the inlet vial is changed with vial containing low pH electrolyte with mobilization agent, e.g. SDS, and then both the mobilization and separation is proceeded (mobilization part).
In our contribution, a proposition of mechanism of the accumulation part based on mathematical simulations in Simul program [1] and experiments with model systems employing contactless conductivity detection [2] will be shown. The study of the mobilization part will be also covered [3]. Some of the applications of the on-line accumulation/mobilization preconcentration technique will be discussed.

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The first Experience with Mutation Scanning using High-Resolution Melting Analysis : NF1 gene

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High resolution melt curve analysis (HRM) is a simple post-PCR technique which can be used for mutation scanning. The technique requires the use of standard PCR reagents only and the fluorescent intercalating dsDNA binding dye. The specific dye allows to detect heteroduplexes arised during PCR. Sequence variations are detected by changes in the shape of the melting curve compared to a negative control. The changes in PCR conditions due to fluorescent dye added seem to be the only disadvantage, the reoptimalization of PCR conditions used by other detection systems are necessary.
At the first stage, PCR conditions for 20 shortest amplicons, used on DHPLC method before, were reoptimalized. Annealing temperature and Mg^{2+} PCR gradient were a useful tool for PCR conditions optimalization. Details and difficulties of optimalization will be presented. For verification of regular detection we have used 24 control samples carrying sequence variations

obtained a different method. In all these cases the positive control has been reliably identified.

Our recent study indicantes that HRM could have a mutation detection sensitivity which is comparable to currently available scanning techniques as DHPLC.

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High-performance size-exclusion chromatographic and spectrometric analysis of dissolved organic matter in lakewater samples

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Dissolved organic matter (DOM) plays a significant role in aquatic ecosystem influencing availbility of nutrients or contaminants as well as affecting carbon cycling. DOM consists mainly of humic substances, proteins, carbohydrates and other macromolecules which are formed from the decomposition of microbial cellular material and higher plant detritus.

The investigation of aquatic biomolecules gives an overview of the processes going on in the studied area (polymerisation, degradation, etc.). It is important for the understanding of biochemical and geological processes and estimation of the environmental conditions in water systems.

The aim of the present study was to characterise aquatic DOM from Lake Rõuge Tõugjärv, South-Estonia, using high-performance size-exclusion chromatography (HPSEC), laser desorption/ionisation time-of-flight mass spectrometry (LDI-TOF MS) and fluorescence spectrometry. To reveal the possible changes in the lake environment samples taken from the inflow, central and outflow points of the lake were analysed. The vertical distribution of aquatic DOM constituents was estimated by analysing lakewater at several depths from surface, namely 0.5 m, 10 m and above the sediment.

HPSEC analysis revealed the presence of fraction which was related to humic substances (HS). HS fraction was present in all lakewater samples. In addition, inflowing water contained high-molecular weight fraction which could correspond to proteinaceous matter. Fluorescence spectrometry results were in a good agreement with those obtained by HPSEC. The weight- and number-average molecular weights of the lake water DOM were found to be 1900 and 600, respectively. LDI-TOF MS revealed several groups of humic substances with the molecular masses of 300-500, 585-740, 760-900, and 1000-1100.

Enhanced resolution of the sample constituents by ITP-CZE with column-coupling based on the use of discrete spacers

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Capillary zone electrophoresis (CZE) with isotachophoresis (ITP) in a column-coupling technique (ITP-CZE) was investigated to use of discrete spacers (DSs) as loadable into the samples of complex ionic mixtures. Here, the DSs were chosen to form the ITP zones by the leading and terminating ions and transferred these zones to the CZE stage. In addition, the leading ion was essential to transport outside from the separation system as using the ITP outlet. Due to this, the CZE stage provided (1) the ITP zones for a set of the mobility subintervals and (2) the sample constituents migrated in the boundary layers as formed by the ITP zones (DSs).
In our study were included ITP-CZE (without the DSs) and ITP-DS-CZE (with the DSs) while operated for computer simulations (SIMUL, B. Gaš et al.). For example, these simulations were taken for 40 model analytes with and without three DSs. In fact, the resolutions of these model analytes were enhanced by ITP-DS-CZE, especially, when compared for ITP-CZE.
Our experiments for ITP-DS-CZE and ITP-CZE, in the cationic mode, were carried out for complex human urine samples. Clearly, these experiments were significantly enhanced the resolutions for ITP-DS-CZE while compared for ITP-CZE.
In fact, using the simulation and experimental investigations, increased for the second dimensions by ITP-DS-CZE. Still, these ITP-DS-CZE simulations,

as operating for different model analytes, showed some logical restrictions for the second dimensions.

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Simple and Fast Determination of Ammonium in Wastewaters by Electrophoresis Chip with Column-Coupling and Conductivity Detection

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Analytical potentialities of capillary electrophoresis (CE) for the determination of ammonium in wastewaters on the column-coupling (CC) chip with conductivity detection were investigated in this work. Capillary zone electrophoresis (CZE) with the electric field and/or isotachophoresis (ITP) sample stacking was employed for pre-concentration on the CC chip. The background electrolytes were developed as including acetic acid (pH ≈ 3) and also tartrate and electro-neutral (18-crown-6-ether) ligands. Rapid CZE and ITP-CZE were found for ammonium as resolving for typical cations (e.g., sodium, potassium, calcium and magnesium) present in wastewaters. Under preferred working conditions (hydrodynamically closed separation system and suppressed electroosmotic flow), both employed methods provided very good repeatabilities of the migration (0.2-0.8 % RSD values for the migration time) and quantitative (0.3-4.9 % RSD values for the peak area) parameters in the model and wastewater samples. A 20 μg/l concentration limit of detection (cLOD) for ammonium was found in the CZE separations. On the other hand, this analyte was found for a 40 μg/l cLOD in the ITP-CZE separations. It should be also mentioned that both methods loaded a 900 nl sample injection volume on the chip. Very good agreements for the determinations of ammonium, covering different wastewaters, were shown for both employed chip methods. These methods included only a minimum

sample pre-treatment (dilution and filtration). In addition, the employed chip methods and spectrometry (ISO 7150-1 method, see this reference) agreed very well in the determination of ammonium in tested wastewater samples. In fact, all of these methods found ammonium at 9-46 mg/l concentrations in a set of various wastewater samples.

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ISO 7150-1, Water quality - Determination of ammonium - Part 1: Manual spectrometric method, ISO, Geneva, 1984.

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Combination of polydimethylsiloxane microchip with external contactless conductivity detector

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Microchip electrophoresis, which is characterized by very fast analysis and by low consumption of sample and reagents, is very promising tool for fast and reliable determinations of biologically active low-molecular weight substances (amino acids, organic acids, amines) in body fluids. We have developed a technology for fabrication of polydimethylsiloxane microchips with the specific design of separation channels, which enables separation of such

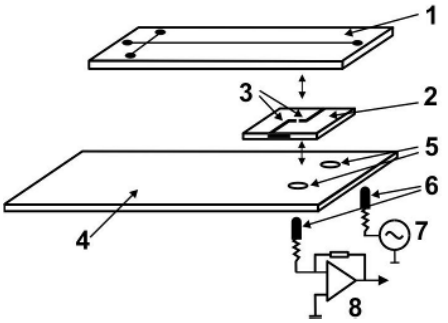

Figure 1. Polydimethylsiloxane microchip combination with external contactless conductivity detector. 1 – microchip, 2 – ceramic plate, 3 – electrodes (gold films), 4 – plexiglas plate, 5 – holes for contacts, 6 – flexible contacts, 7 – generator, 7 – electronic circuitry.

mixtures such as body fluids. Since most of low-molecular bioactive compounds is transparent to UV radiation, an electrochemical detection was used for their determination [1,2]. A novel combination of external contacless conductivity detector and polydimethylsiloxane microchip will be presented. Miniature planar electrodes were created on the surface of ceramic plate. The high adhesive strength of polydimethylsiloxane to the surface of planar electrodes ensured a good capacity coupling between the electrodes and the solution in the channel. Several applications of this approach in the separation of mixtures of bioactive molecules will be discussed.

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Development of microfluidic systems for multidimensional separations

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Combinations of electrophoretic or chromatographic techniques (zone electrophoresis, isotachophoresis, isoelectric focusing) with electrospray mass spectrometry are of great practical interest for high resolution separations. The use of focusing techniques (ITP, IEF) can provide higher loading capacity resulting in better concentration sensitivity. The final separation step coupled on-line with mass spectrometry then leads to a high performance system with ultimate resolution. A microdevice combining low dead volumes between interconnecting channels as well as minimization of

the liquid flow related band broadening effects is one of the instrumental approaches. The devices under development are fabricated in glass using photolithography, wet chemical etching and thermal bonding. For repeatable use the sandwich design with electrode chambers and sample inlet ports located on an external plastic manifold was selected. Additional practical parameters also include the channel surface modifications, which can influence the sample sorption or act as a stationary phase with a potential for chromatographic separation mode. The presented system is designed for focusing relatively large sample volume first in a wider channel followed by the separation in the second narrow channel connected to an integrated liquid junction based interface with pneumatic nebulizer for on-line electrospray coupling with mass spectrometry.

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Preparation and Physico-chemical Properties of CdTe Quantum Dots

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There is a big growth of papers describing the implementation of quantum dots as a fluorescent probes in chemistry, cell biology and medicine during the last years [1]. The main advantages of quantum dots when compared to the conventional organic fluorescent dyes are high quantum yields, practically no photobleaching, wide range of excitation wavelengths and narrow emission spectra [2, 3].

We have prepared water-soluble CdTe quantum dots by the chemical reaction between cadmium chloride and sodium hydrogen telluride at the presence of 3-mercaptopropionic acid [4]. Their fluorescence emission maximum is 600 nm with the bandwidth of 58 nm at the half height of its intensity. Thus, the particle size determining the wavelength of the

fluorescence emission was evaluated to be 3.5 nm. The extraordinary broad excitation spectrum lies in the range from 300 to 550 nm with the absorbance maximum at 469 nm. The lifetime of the fluorescence, determined by the time-resolved fluorescence spectrometry, is 15.3 ns.

The capability of the prepared quantum dots to be used as a non-selective fluorescent labeling of yeast or human lymphocyte cells has been tested. The efficient uptake of these nano-particles into the living cells is apparent after 30 min under the epifluorescence microscope. This kind of labeling has a potential for single-cell analyses with LIF detection. Another promising property of quantum dots is their easy conjugation to antibodies, enzymes and other functional proteins. This allows for the selective immuno-labeling and localization of specific molecules at the surface or even in the interior of cells. This way, the FAS and FADD proteins, active as membrane receptors in the apoptotic signal pathways of lymphocytes, can be visualized as a stable fluorescence signal under the microscope.

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Search for the posttranslational modifications of barley proteins during malting by IEF followed by MS identification

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The study of barley proteins and their changes during malting is a task demanding proper separation technique prior to the MS identification of proteins. During malting process the barley proteins are gradually cleaved and modified. Huge amount of lower sugars released from starch and increased temperature cause glycation of lysines in proteins. Glycation changes the molecular weight of the protein just slightly. On the other hand, glycation causes the change of isoelectric point (p*I*) value of the protein. Wherefore, the isoelectric focusing (IEF) on gel was chosen as a method, which can separate proteins with high resolution. Moreover, analytes with tight p*I* value of such as proteins are during IEF concentrated into very narrow zones.

The calibration of pH gradient is crucial since the sample itself and salts in it may affect the pH gradient significantly. It is necessary to add calibrant not interacting with analyzed proteins directly to the sample. In the suggested method the low-molecular mass organic compounds are used as p*I* markers. After excision, the p*I* markers are washed from the gel prior to the protein digestion. The identification of the p*I* markers by MS provides the information about the accurate pH value of the gel piece.

Identification of protein in more then one gel piece or in the gel pieces with the p*I* markers non-corresponding to the calculated p*I* of these proteins can indicate the possible posttranslational modification (PTM). In case of malt, protein Z with 22 lysines in its structure is a good example for study of glycated proteins. With our method, protein Z was identified in the gel piece with the p*I* markers corresponding to the calculated p*I* of this protein, but also in the gel pieces with the p*I* markers of lower p*I* than is the calculated p*I* of protein Z. The glycation of these forms was confirmed by MS/MS of glycated peptides.

Since the 3/10 pH gels showed a huge quantity of proteins and especially glycated proteins in the pH region approximately about 4.5 to 7, the 4/6 pH gradient was used for deeper proteomic study of barley grain and malt. The differences in protein profile between barley grain and malt as well as intervarietal differences were observed.

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How the barley malting changes the protein profile?

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The cereal seed proteins have been one of the main topic of research for many years, with aim of understanding their structures, control of synthesis and role in grain utilization. Barley (*Hordeum vulgare*) proteome analysis is important especially for food and brewing industry.

The effect of malting on the content and the degree of posttranslational modification of barley proteins was studied. Three different varieties of malted barley were used for this research. The malt samples were taken in the course of malting (from barley grain to complete malt). The amount and composition of water soluble proteins have crucial influence on the suitability of grains of individual barley cultivars for its final uses. Therefore the changes in their profile were compared by using 2-D GE and MALDI-TOF mass spectrometry.

The special attention was paid to the investigation of protein glycations. This term summarizes non-enzymatic reactions between amino groups of proteins and free reduce carbohydrates. The analysis of intact proteins by MALDI-TOF mass spectrometry revealed significant influence of malting processes

on the degree of glycation of barley lipid transfer protein (LTP1, 9 kDa) and showed the other differences between barley grains and malt. These changes are important, because some of modified proteins cause haze in beer while others are important for foam formation and stabilization.

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AERODYNAMIC SIMULATION OF ESI-MS INTERFACE USING THE FINITE ELEMENTS METHOD

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Electrospray ionization is a widely used soft ionization technique in mass spectrometry especially for on-line coupling with separations and for analysis of large molecules. The instrumentation miniaturization and integration are today's most demanding ambitions, since faster analyses come along with savings in analysis time and mostly also with improvement in sensitivity. To place an aerodynamic interface in front of the Mass Spectrometer is one of the possible strategies how to deal with the problems of spraying from a microdevice. The interface is expected to:
- enable spraying from tipless microdevices (separations chips)
- improve sampling efficiency
This presentation will describe a numerical model based on the Finite Elements Method which was developed to do preliminary estimates of the sampling efficiency of an interface design prior its fabrication.

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On-target (time-resolved) laser induced fluorescence detection

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Protein detection and identification is a task commonly performed by a gel electrophoresis in combination with in-gel enzyme digestion and mass spectrometry (MS). An alternative approach utilizes micro-column separation techniques, such as a capillary electrophoresis (CE) or a nano-liquid chromatography (LC), in *on-line* coupling with spray ionization techniques. A different approach to examine biomolecules engages a platform layout where the micro-column separation technique may be linked *off-line* to a range of detection modes, such as MALDI MS along with enzymatic digestion or laser induced native fluorescence (LINF), correspondingly, time-resolved laser induced native fluorescence (TR-LINF). A MALDI target, sited in a partially evacuated chamber, acts exactly as the introduced platform for the modes mentioned above.

The linkage between separation and infusion capillary is carried out by a liquid junction, which allows keeping the separation efficiency unaffected. The flow of the auxiliary liquid is induced by a sub-atmospheric pressure, which is about 20 kPa. The probe with infusion capillary is operated to generate discrete fractions of influent along the MALDI target[1].

Generally, native fluorescence of proteins is based on fluorescence of three amino acids, tryptophan, tyrosine and phenylalanine, whose excitation bands are situated in UV region. Contribution of these amino acids to the fluorescence yields is not evenly balanced and, as indicated by chemical structures, the tryptophan provides the highest fluorescence yield.[2] Hence, the overall amount of tryptophans in the protein molecule plays an indispensable role for the appropriate native fluorescence detection.[3] Involving TR-LINF to the comprehensive study of biomolecules allows addition of another dimension for gaining further information of particular proteins. In case of a poor signal to noise ratio or immoderate scattering, TR-LINF might be a more advantageous detection technique.

In this contribution, attention was paid to investigate factors affecting the signal to noise ratio and scattering in order to optimize the method. In the case of LINF, the work was focused on the use of proper MALDI target cleaning procedures. This involved use of hydrogen peroxide for complete oxidation of organic residues, or spin-coating procedure to create a uniform layer minimizing irregularities of the MALDI target. The TR-LINF detection optimization concerned with the proper decay time selection and a possibility to automate the signal acquisition of the entire separation record. Examples of decay times were given for selected compounds and a model experiment comparing LINF and TR-LINF acquisition was demonstrated.

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Laser diode (405 nm) as an excitation source in capillary electrophoresis with laser-induced fluorescence detection analysis of GFP-tagged proteins.

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Green fluorescent protein (GFP), discovered by Shimomura et al. in 1960s (1), is one of the most widely studied and exploited proteins in biochemistry and cell biology as a marker of gene expression and protein targeting in intact cells and organisms.

The wild-type protein is characterized by a major excitation peak at 395 nm, which is about three times higher than the minor peak at 475 nm. The reason is that only 15% of the protein has the deprotonated or anionic chromophore that absorbs at 475 nm.(2,3) Excitation at 395 nm gives emission peaking at 508 nm, whereas

excitation at 475 nm yields a maximum at 503 nm. Ar+ laser (488 nm) is a commonly used excitation source for capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) analysis of GFP and GFP-tagged proteins mainly for its availability in chemical laboratories all over the world.

In this work, using laser diode (405 nm) as a prospective excitation source for monitoring of phosphorylation process of cytosolic histidine-containing phosphotransfer proteins (designated as AHP1-6 in Arabidopsis) has been suggested. The main advantage of CE-LIF method, in comparison to SDS PAGE, is the possibility of preservation of native conditions required for discrimination between phosphorylated and non-phosphorylated state of AHP. To our knowledge, this laser emitting at 405 nm has not been applied for CE-LIF of GFP and/or GFP-tagged proteins yet.

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Proteolytic digestion of hordeins with proline-specific protease from *Aspergillus niger* under nonreducing and reducing conditions

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One of the important proteins in barley are hordeins. These proteins soluble in alcohol mixtures with water (e.g. 60% ethanol) form about the half of the protein composition of the barley grain. They function mainly as storage

proteins and contain mostly proline and glutamine amino acids, which sometimes form the toxic sequences for patients with celiac diseases. Hordeins also cause the formation of the haze in the beer due to their coagulation with polyphenolic substances contained in the malt.

Commercially available enzyme isolated from *Aspergillus niger* is an endoprotease with enzymatic specificity at the carboxy side of the proline amino acid. Although this enzyme shows cleavage specificity for some other amino acids, it is suitable for treatment of the malt for removing of hordeins during the production of beer.

Extracted hordeins were digested with proline-specific protease from *Aspergillus niger* both under nonreducing (without DTT treatment) and reducing (with DTT treatment) conditions. Mixtures of digested hordeins were separated using 1D SDS PAGE and the separated protein bands were submitted for in-gel enzymatic cleavage and MALDI-TOF/TOF mass spectrometry analysis. The results showed that the most hordeins are fully digested with proline-specific protease even under nonreducing conditions.

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Nucleotide analysis in cultured skin fibroblasts by capillary electrophoresis

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Metabolomic analysis of human cultured skin fibroblasts (hCSF) is difficult task due to limited number of cells available compared to metabolomic experiments performed on microbes. Under usual culture conditions less

then 1 million cells in 25 cm2 flask is obtained, corresponding to a total cellular volume of approximately 1 µl. To date, only one paper dealing with metabolomic analysis of fibroblasts has been published (Munger J., 2006). The aim of our work was to adopt previously published method (Friedecky D., 2007) for analysis of intracellular nucleotide content in hCSF.

Fibroblasts were cultured using standard protocols in Quantum 333 medium (PAA laboratories, AT) + 1 % fetal bovine serum. Cells were harvested by brief trypsinisation. In initial experiments repeatability culture-to-culture of nucleotide concentrations was tested in seven parallel cultures. Relative standard deviations for individual nucleotides range from 13% to 31% for ATP and ITP, respectively. Quenching and extracting nucleotides using previously published method (Munger J., 2006) by methanol:water (80:20) solution at -40°C provided unusual adenylate energy charges. In further extraction experiments (subsequent water and trichloroacetic acid extraction of pellet) we observed that nucleotides are not fully recovered from fibroblasts pellet by methanol:water approach. Recovery was 57%, 51% and 98% for ITP, ATP and NAD, respectively. We finally used methanol:water (2x), followed by water and 0,2 M trichloroacetic acid for extraction of hCSF.

Analysis of intracellular nucleotides was accomplished by capillary electrophoresis using running buffer 40 mmol/l citrate - gama-aminobutyric acid (pH 4.3) with 0.8 mmol/l cetyltrimethylammonium bromide. Average separation efficiency was 310 000 theoretical plates/m and limits of detection in the range 0,7 - 1,4 micromol/l.

In conclusion capillary elecrophoresis allows analysis of seven most important nucleotides in hCSF. Methanol:water extraction, frequently employed in metabolomic studies, is not useful in analysis of nucleotides in hCSF.

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Determination of ITPase activity in dry blood spots by capillary electrophoresis

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Background: Inosine triphosphate pyrophosphohydrolase (ITPase) is involved in metabolism of the thiopurine drugs and its deficiency is associated with thiopurine intolerance. The aim of this study was to develop method for the determination of ITPase activity in dry blood spots.

Methods: Dry blood spots were used for the assay. Samples were incubated with ITP and the enzymatic conversion to inosine monophosphate (IMP) was terminated by trichloracetic acid. The IMP was quantified at 250 nm by capillary electrophoresis using a buffer consisting of citric acid (40 mmol/L) and cetyltrimethylammonium bromide (0.8 mmol/L) adjusted with gamma-aminobutyric acid to pH 4.4.

Results: The method is linear up to 10 mmol/L with limit of detection for IMP of 6.9 μmol/L (~ activity of 3.6 μmol IMP/(g Hb.h)). Analysis time was 0.8 min. Imprecisions measured using IMP-enriched samples were 2.1, 1.2, and 1.0 % (within-day CV) and 4.2, 3.2, and 2.4 % (between-day CV) for 0.06, 0.54, and 3.00 mmol/L addition of IMP, respectively. The reference values for healthy Caucasian blood donors were 48 - 404 μmol IMP/(g Hb.h). Comparison using Bland-Altman test revealed usefulness of dry blood spots for the enzyme assay.

Conclusion: Capillary electrophoresis provides a high-throughput tool for the determination of ITPase activity in dry blood spots aimed at the prediction of toxicity during thiopurine therapy.

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Determination of thiopurine methyltransferase activity by capillary electrophresis

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Background: Thiopurine methyltransferase (TPMT) catalyzes the methylation of thiopurine drugs (e.g. 6-mercaptopurine, azathioprine) used for treatment of leukemia and inflammatory diseases. Decreased activity of TPMT is associated with hematopoietic toxicity after administration of standard doses of the drugs.

Methods: Lysed erythrocytes were incubated with mercaptopurine and the enzymatic conversion to 6-methylmercaptopurine (6-mMP) was terminated by trichloracetic acid. Final conditions consisted of CAPS buffer (100 mmol/L) adjusted with triethylamine to pH 11.2 with addition of 10 % methanol, electric field of 740 V/cm, 25 °C, hydrodynamic injection of 6 s, detection at 294 nm.

Result: Limit of quantification of the method is 2.5 μmol/L (S/N=10), which corresponds to enzyme activity 9.3 nmol 6-mMP / (gHb.h). Reference values were fully comparable with previously published HPLC assays.

Conclusions: We developed capillary electrophoretic method for determination of TPMT enzyme activity in erythrocytes. The method can be used for prediction of adverse reaction to thiopurine therapy.

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Determination of Imatinib in plasma by capillary electrophoresis

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Background: Imatinib mesylate (Glivec, Gleevec, Novartis Pharma AG) is a new drug used for targeted treatment in haematology and oncology. It belongs to a class of agents called signal transduction inhibitors, which interfere with the signal pathways that govern the growth of tumour cells. Resistance to the therapy is reported as the most serious problem. Inclusion of plasma drug levels measurement is important for successful management of resistance.

Methods: Samples were prepared by precipitating the plasma proteins with methanol. Separation conditions were optimized in respect to pH, composition of background electrolyte and electrophoretic conditions.

Results: Final analytical conditions were as follows: 60 mmol/L citrate buffer adjusted with □-amino-n-butyric acid to pH 3.6, 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 8.10^{-8} mol/L (S/N=3) due to high absorption coefficient. The plasma imatinib concentration were measured in 50 treated patients with chronic myeloid leukemia and were in the range of 0.53 – 18.84 μmol/L. No interferences were observed in all samples analyzed.

Conclusions: We report here a capillary electrophoretic method for determination of imatinib in plasma allowing analysis with sensitivity sufficient for clinical settings.

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Analysis of adenine nucleotides and nicotinamide coenzymes by CZE in combination with field enhanced stacking.

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Being the intermediates of biochemical reactions, metabolites play a very important role in connecting the many different pathways that operate within living cell. Among them adenine nucleotides and nicotinamide coenzymes are central energetic metabolites in this complex metabolic network. Determination of these metabolites is thus important for metabolomic studies, because their pool analysis characterize the energetic state of the cell under a variety of physiological conditions during cell growth. As the importance of metabolome analysis was recognized, several metabolite analysis methods by means of GC-MS, LC-MS, NMR and FT-ICRMS have been developed. In addition to these well established methods capillary electrophoresis (CE) is gaining the position in this field.

A method for determination of adenine nucleotides (ATP, ADP, AMP) and nicotinamide coenzymes (NAD^+, NADH, $NADP^+$, NADPH) by CE was developed. As low concentrations of these metabolites are presented in the cellular extracts the on-line preconcentration technique – field enhanced stacking – was combined with capillary zone electrophoresis (CZE) to enhance the concentration sensitivities. The determination was performed in a 75 μm fused silica capillary using separation voltage 18 kV (positive polarity), temperature of capillary 20 °C and direct detection at 260 and 340 nm. 100 mM ammonium carbonate buffer (pH 9,6) was used as the background electrolyte, but addition of beta-cyclodextrin up to 5 mM concentration was required for better resolution of given analytes. Metabolites samples were dissolved in deionised water and injected into the capillary hydrodynamically with a pressure of 35 mbar for 20 s. Under these conditions, the detection limits were in the range of 300 – 400 nM at a signal-to-noise ratio (S/N) of 3. The optimized methodology was applied on the cell extract of *Paracoccus denitrificans*. The concentrations of given metabolites in the bacterial cells under different growth conditions were determined.

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Rigorous Statistical Analysis of Mobility Curves for the Determination of pK_B of Weak Bases

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This contribution brings a new rigorous and complete statistical approach to the data processing of the mobility curves of univalent weak bases. It is based on application of least square method to the equation of the related mobility curve. Thus, an equation for the best fit is derived and its mathematical solution is found. The solution brings best estimates of the mobility curve parameters, i.e., dissociation constant K and ionic mobility of protonated base U. Further, explicit formulas have been derived for the calculation of related statistical parameters, i.e., standard deviations of effective mobility $s_{u,}$ of the dissociation constant s_K, and of ionic mobility of protonated base s_U. The mathematical procedure presented here does not impose any limitations upon the numerical data used, i.e., the experimental values of mobilities and pH may be real numbers (positive, negative, zero).

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