# CECE 2011

"... bringing
people and ideas
together ..."

## 8th International Interdisciplinary Meeting on Bioanalysis

November 3-4, 2011
Hotel Continental, Brno, Czech Republic
www.ce-ce.org

iac brno

**Organized by:**
**Institute of Analytical Chemistry AS CR, v. v. i., Veveří 97, 602 00 Brno**

Organizing committee: František Foret, Ludmila Křivánková, Karel Klepárník, Iveta Drobníková
Webmaster: František Matulík

**Find the meeting history and more at www.ce-ce.org**


# ISBN 978-80-904959-0-6

# Foreword

Welcome to CECE 2011. After two years in Pecs, Hungary we are back to Brno. I want to thank Professor Ferenc Kilár for his hospitality and excellent organization of the meeting in 2009 and 2010 and hope we will find new international venues in the future again. 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 8$^{th}$ year of CECE. Today, the two day meeting with invited lectures and poster sessions is still 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 the invited speakers and all the participants and hope that you will enjoy the scientific presentations as well as personal contacts and informal discussions.

*Franta Foret*

# Program

**Thursday, November 3**

9:00 – 9:15 **Conference opening**

9:15 – 9:45
**Detlev Belder**, Universität Leipzig, Germany
Enantioselective catalysis and analysis in single microfluidic devices

9:45 - 10:15
**Christian Klampfl**, Johannes Kepler-University Linz, Austria
Coupling CE to MS: new interfaces, new ion sources new applications

10:15 – 10:45
**Oliver Trapp**, Ruprecht-Karls-Universität Heidelberg, Germany
CE as Tool to Investigate the Stereodynamics and Catalytic Reactions

10:45 – 11:15
**Ziad El Rassi**, Oklahoma State University, Stillwater, USA
Immuno-Monoliths at Reduced Nonspecific Interactions

11:15 – 11:45
**Lloyd M. Smith**, University of Wisconsin-Madison, USA
New Technologies for the Genome Age

11:45 – 14:00 **Lunch break – poster session**

14:00 – 14:30
**Jiri Fajkus**, Masaryk University Brno, Czech Republic
Analysis of telomeres and telomerases

14:30 – 15:00
**Rui Vitorino**, University of Aveiro, Portugal
What is saliva? A perspective of salivary proteomics at University of Aveiro

15:00 – 15:30
**Françoise Nepveu**, University of Toulouse 3, France
Indolone-n-oxides: relation between redox properties and antimalarial activities.

15:30 – 16:00
**Pier Giorgio Righetti**, Politecnico di Milano, Italy
The proteome Argonauts: conquering the "golden fleece" of alcoholic beverages and soft drinks via combinatorial peptide ligands

19:00
**Conference dinner with traditional Moravian music-Hotel Continental**

**Friday, November 4**

9:00 – 9:30
**Huan-Tsung Chang**, National Taiwan University, Taiwan
Separation of DNA and DNA-templated metal clusters by CE-LIF

9:30 – 10:00
**Andras Guttman**, University of Debrecen, Hungary
Capillary electrophoresis analysis of the altered glycosylation of immunoglobulins in autoimmune diseases

10:00 – 10:30
**Aran Paulus**, Bio-Rad Laboratories, USA
Microfabricated and Lab-on-a-chip Devices in the Life Science Industry: Promises, Products and Problems

10:30 – 11:00 **coffee break**

11:00 – 11:30
**Steven Soper**, University of North Carolina, USA
Single-Molecule Electrochromatography in Nano-columns with Conductivity Readout: A Novel Approach for High Throughput DNA Sequencing

11:30 - 12:00
**Andreas Manz**, KIST Europe, Saarbrücken, Germany
Microfluidics for applications in chemistry

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

14:00 – 14:30
**Ferenc Kilar,** University of Pecs, Hungary

Isoelectric focusing coupled to mass spectrometry for bioanalysis

14:30 – 15:00
**Gyula Vigh**, Texas A&M University, USA
Design, Synthesis and Analytical Characterization of a New Family of pI Markers

15:00 – 15:30
**Vaclav Kasicka** Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic
Investigation of biopeptide interactions by capillary electrophoresis

15:30 **Closing remarks**

# About the Invited Speakers

**Detlev Belder**
Universität Leipzig, Lehrstuhl für Analytische Chemie, Johannisallee 29, 04103 Leipzig, Germany
http://www.belder.de

Prof. Belder studied chemistry in Clausthal and Marburg: he obtained his PhD in Analytical chemistry in 1994. After a Postdoc stay at SmithKline-Beecham in England he worked for 11 years (1995-2006) as the head of the separation science department at the Max-Planck-Institut für Kohlenforschung (Mülheim an der Ruhr). In 2006 he accepted the offer from the University of Regensburg as an associate professor in Analytical Chemistry. Shortly afterwards he moved in 2007 to the University of Leipzig accepting the offer for a chair in Analytical Chemistry. Prof. Belders current main research interest is the development of microfluidic lab-on-a-chip devices in Analytical and also in Synthetic Chemistry.
Examples for his research topics are:

- Microfabrication
- Chip Electrophoresis
- Chip Chromatography
- Chiral Separations
- Coupling Microfluidic Chips with Massspectrometry
- Surface Chemistry and Coatings
- Enantioselective Catalysis on Chip
- Integration of Synthesis and Analysis on a single device
- Fluorescence Detection and Microscopy
- Raman Detection and Microscopy
- Microfluidic Sensors

Recent publications:

*Asymmetric Organocatalysis and Analysis on a Single Microfluidic Nanospray Chip*
S. Fritzsche, S. Ohla, P. Glaser, D. S. Giera, M. Sickert, C. Schneider, and D. Belder, *Angew. Chem. Int. Ed*., **2011**, DOI: 10.1002/anie.201102331

*Microfluidic chips for chirality exploration*
S. Nagl, P. Schulze, S. Ohla, R. Beyreiss, L. Gitlin, D. Belder, *Anal. Chem*. **2011**, 83, 3232–3238.

*PDMS free-flow electrophoresis chips with integrated partitioning bars for bubble segregation*
S. Köhler, C. Weilbeer, S. Howitz, H. Becker, V. Beushausen and Detlev Belder, *Lab Chip*, **2011**, 11, 309-314.

## Huan-Tsung Chang

**(**Department of Chemistry National Taiwan University, http://www.ch.ntu.edu.tw/~htchang/) focuses on development of micro-nano techniques and green approaches. He has demonstrated a number of new concepts and methods, including (1) On-line concentration and separation capillary electrophoresis (CE); (2) Nanoparticles (NPs) filled capillary electrophoresis (NFCE); (3) Green syntheses of anisotropic structures of metallic NPs, quantum dots (QDs), and Te/Au nanowires in aqueous solution; (4) NPs as concentration and laser desorption/ionization matrices in mass spectrometry; (5) Aptamers functionalized NPs for the detection of metal ions, small molecules, and proteins; (6) Preparation and applications of fluorescent gold nanodots (Au NDs); (7) Sensitive and selective DNA probes for the detection of DNA and metal ions; (8) Preparation of fluorescent DNA-templated metal clusters for the detection of metal ions and DNA; (9) DNA-functional nanomaterials for control of enzyme activity; (10) Fabrication of quantum dot sensitized solar cells; (11) Pt nanomaterials for highly electrocatalytic methanol oxidation; and (12) New nanomaterials (Te/Au nanowires) as substrates for surface enhanced Raman scattering. He has published more than 180 papers.

**Recognition:** Young Chemists Award of the Chemical Society Located in Taipei (2000); Young Scholar Award, College of Science, National Taiwan University (2000); Fu Szu-Nien Award, National Taiwan University (2005); Outstanding Research Award, National Science Council, Taiwan (2007)**;** Y. Z. Hsu Scientific Paper Award (Green Technology Category) (2008)**;** Distinguished Professor of National Taiwan University (2008-present)

**Jiří Fajkus** (http://www.ceitec.eu/contact/research-programmes/genomics-and-proteomics-of-plant-systems/) has started research during his PhD studies at the Institute of Biophysics, ASCR (1988-1992) with analysis of structure and function of plant DNA repeats and their chromatin structure at the level of nucleosomes and chromatin loops. After his postdoctoral stay in the laboratory of Prof. Ronald Hancock, Laval University Cancer Research Centre, Québec, Canada, where he has been performing mapping of topoII-associated chromatin regions in the human *hprt* gene (1992-1993,1995), he started his own research group at the Institute of Biophysics. He became interested in biology of telomeres, and plant telomeres in particular. In 1995, he has described a specific nucleosome structure of plant telomeres, later on developed into a columnar model of telomeric chromatin (in collaboration with Prof. Edward N. Trifonov, 2001). In 1996 he was the first to detect telomerase in plant cells. In 1998, his group described the maintenance of telomere length stability during plant development and a reversible

control of plant telomerase activity, the features distinguishing telomere biology of plants from that of humans. Since 2000, he has published a number of studies on plant telomere binding proteins and their interactions, molecular evolution of plant telomeres and telomerases, and also on applications of telomere and telomerase analysis in oncology diagnostics. For the purpose of diagnostic applications, he has developed a two-color quantitative real-time version of TRAP assay for telomerase activity (2003). His recent achievements include detection of telomerase-independent telomere lengthening in plants and its role in plant development (2008), description of the selective loss of telomeres and rDNA genes in *Arabidopsis thaliana CAF1* (chromatin-assembly factor 1) mutants (2010), or studies on the epigenetic regulation of telomere maintenance (2011).

**Recognitions**

- Prize of the Rector of the Masaryk University and J. E. Purkinje Medal - 1987;
- Award of the Czech Ministry of Education "Talent 98" - 1999;
- Prize of the Minister of Education for Research – 2000,
- Otto Wichterle Premium (Czech Academy of Sciences) - 2002.

**András Guttman** is a research professor at the Barnett Institute in Northeastern University (Boston, MA), also heading the Horváth Laboratory of Bioseparation Sciences (HLBS) in University of Debrecen (Hungary) and was recently named as Marie Curie Chair Professor by the European Commission. His fields of research interest are glycomics, biomarker discovery and microfabricated device technology. Professor Guttman previously held industrial research positions at Novartis (La Jolla, CA), Genetic Biosystems (San Diego, CA) and Beckman Instruments (Fulleton, CA). He has contributed more than 200 scientific publications, 30 book chapters, edited several textbooks and holds 18 patents. He is the president of the Hungarian Chapter of the American Chemical Society, board member of CASSS and on the editorial panels 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.

## Václav Kašička

(http://www.uochb.cz/web/structure/471.html) received MSc. degree in 1977 and the title RNDr. (Rerum Naturalium Doctor) in 1979, both of them in physical chemistry at Faculty of Science, Charles University, Prague, and the CSc. (PhD.) degree in biochemistry in 1985 in the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague. In this Institute, he is currently head of the Laboratory of Electromigration Methods. His working interests involve research and development of theory, instrumentation and methodology of capillary and free-flow electroseparation methods and their applications to analysis, purification and physicochemical and biochemical characterization of amino acids, peptides, proteins and other biomolecules as well as functional organic molecules. V. Kašička is author or co-author of 110 papers in international peer-reviewed journals with more than 1 000 citations without self-citations according to ISI. Further, he is author or co-author of about 40 papers in the proceedings of international symposia, 15 chapters in books and textbooks, 25 patent pendings and more than 100 lectures at international symposia.

V. Kašička is one of the editors of the Journal of Separation Science, editor of special issues of Electrophoresis, J. Sep. Sci. and J. Chromatogr. B., and member of editorial boards of international journals, e.g. Electrophoresis, Journal of Separation Science, Current Analytical Chemistry and The Open Nanoscience Journal. V. Kašička is member of permanent Scientific Committee of the series of the International Symposia on Electro- and Liquid Phase-separation Techniques. He is chairman of the Chromatography and Electrophoresis Group of the Czech Chemical Society and representative of the Czech Republic in the European Society for Separation Science. In 1980-1990-ties, he has been awarded by the prices of the Czechoslovak Chemical Society and Czechoslovak Academy of Sciences for young scientists.

**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 at Pécs. 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. 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).

**Christian W. Klampfl** received his MSc. (1990) and PhD (1993) from the University of Innsbruck/Austria. Moving to the Institute of Analytical Chemistry at the Johannes Kepler University in Linz (where he is currently holding a position as an associate professor) also meant a strong focus on separation techniques. After a year as research associate with Prof. Paul R. Haddad at the University of Tasmania (1999), the author started to conduct first investigations on the hyphenation of capillary electrophoresis with mass spectrometry (CE-MS). The author's interest is still focused on the development of new applications for CE-MS and new methodical approaches such as the combination of microemulsion electrokinetic chromatography (MEEKC) with MS detection. Recently a new field of work namely investigations on novel applications of ambient mass spectrometric techniques has been added to Prof. Klampfl´s portfolio. He has published more than 70 manuscripts and five book chapters in the fields of separation science and is editing special issues of Electrophoresis devoted to the hyphenation of liquid phase separation systems with mass spectrometry on a regular basis.

## Andreas Manz

| | |
|---|---|
| since 2010 | Professor at the Faculty Mechatronics, Saarland University |
| since 2009 | Head of research, Korea Institute of Science and Technology (KIST), Saarbrücken, Germany |
| 2003-08 | Head, ISAS - Institute for Analytical Sciences, Dortmund and Berlin, Germany |
| 2001-08 | Journal founding member and chairman of the editorial board of 'Lab on a Chip' (Royal Society of Chemistry, UK) |
| since 2000 | Member of the permanent scientific committee of MSB (previously HPCE), the International Symposium on Microscale Separations and Analysis |

| | |
|---|---|
| since 1997 | Fellow of the Royal Society of Chemistry (CChem FRSC), U.K. |
| 1995 - 2004 | SmithKline Beecham Professor of Analytical Chemistry at Imperial College, Dept. Chemistry, London, U.K. |
| 1995 - 2000 | Scientific advisor and consultant of Caliper Technologies, Mountain View, California. The company is active in "lab on chip" technology. |
| 1995 | Habilitation thesis (supervisor: Prof. Dr. Grasserbauer) accepted at the Technical University (TU) Vienna, Austria. Topic: 'Micro System Technology for Use in Analytical Chemistry'. |
| 1994 - 2008 | Member of the scientific committee and the board of of µTAS, the International Symposium on Micro Total Analysis Systems |
| 1991 - 1995 | Group leader at Ciba-Geigy Corporate Analytical Research, Basel, Switzerland. |
| 1988 - 1991 | Researcher at Ciba-Geigy Central Research Lab., Basel, Switzerland |
| 1987 - 1988 | Postdoctoral fellow at Hitachi Central Research Lab., Hitachi Ltd., Tokyo, Japan. |
| 1983 - 1986 | Dr. sc. tech. thesis (PhD) under the guidance of Prof. Dr. W. Simon (Dept. for Organic Chemistry, Swiss Federal Institute of Technology, ETH, Zürich, Switzerland). |

## Françoise Nepveu

*Main competencies:*

Molecular properties, analytical chemistry and redox mechanisms for pharmacological applications

*Education:*

1982

Doctorat d'Etat es sciences, University of Toulouse

1976

M2R (DEA) in bioinorganic chemistry, Major of promotion, University of Toulouse

*Positions and employment:*

1988-2010

Professor (PRcex) at the University Toulouse 3

1984-1986

Post-doctoral position, Technische Hochschule Darmastadt, Germany

1982

Post-doctoral position, University of North Carolina, Chapel Hill, USA

1977-1988

Assistant then associate professor

*Publications / Patents:*

115 published articles, 1 patent, 2 book chapters, 30 oral communications.

- Ibrahim H., Pantaleo A., Turrini F., Arese P., Nallet J-P., **Nepveu F.**, Pharmacologicol properties of indolone-n-oxides controlled by a bioreductive transformation in red blood cells, *MedChemComm.*, 2011, DOI: 10.1039/C1MD00127B, new journal (2011) , RCS

- Reybier K., Perio P, Ferry G., Bouajila J, Delagrange Ph;, Boutin J. A., **Nepveu F**. Insights into the redox cycle of human quinone reductase 2. Free Rad Res., 2011, 45(10), 1184-1195
- **Nepveu F**., Kim S., Boyer J., Chatriant O., Ibrahim H., Reybier K., Monje M-C, Chevalley S., Perio P., Lajoie B., Bouajila J., Deharo E., Sauvain M., Tahar R., Basco L., Pantaleo A., Turini F., Arese P., Valentin A., Thompson E., Vivas L., Petit S., Nallet J-P, Synthesis and antiplasmodial activity of new indolon N-oxide derivatives, *J. Med. Chem*, 2010, 53, 699-714**.**
- Ibrahim N., Ibrahim H., Kim S., Nallet J.-P., **Nepveu F**., Interactions between Antimalarial Indolone-*N*-oxide Derivatives and Human Serum Albumin. *Biomacromolecules*, 2010, 11, 3341-3351.
- Reybier K., Ribaut C., Coste A., Launay J., Fabre P.L., **Nepveu F.,** Characterization of oxidative stress in Leishmaniasis-infected or LPS-stimulated macrophages using electrochemical impedance spectroscopy, *Biosensors and Bioelectronics*, 2010, 25(12), 2566-2572.

*Awards, activities, memberships:*

**Awards**: Chevalier dans l'Ordre de la Légion d'Honneur (14/12/2010). Alexander Von Humboldt fellowhip ; PEDR 1990-2010. **Activities**: Head of the laboratory UMR 152 IRD-UT3 2003-2010; teaching activities: 192 h/year in physical chemistry and instrumentation for biological media, drug and food analysis; antioxidants**,** pro-oxidants; supervision of 36 post-graduate students (1986-2005), 13 PhD 1991-2010; 23 grants (1991-2010); Head of the Department of analytical chemistry (1996-2001); President of the local CNU section 39eme (2007-2008): head of laboratories (1991-2002). **Memberships:** Office Member of the Food-Health Consortium of the Region Midi-Pyrenees (2000-2010); member of the Doctoral School in Sciences of Matter; member of scientific societies (SFC, SCT, SFRR, ACS). Expert (AERES, ANR), Reviewer of scientific journals.

**Aran Paulus** is a currently a R&D manager for New Technology and Applications in the Protein Technology R&D group with Bio-Rad Laboratories in Hercules, CA. He holds a Ph.D. in Chemistry from the University of Tübingen, Germany and joined after a post-doc with Prof. Barry Karger at the Barnett Institute of Northeastern University in Boston, MA, the Analytical Research Department of Ciba-Geigy in Basel, Switzerland. His research interests there included capillary electrophoresis for DNA fragments, antisense oligonucleotide analysis and complex carbohydrate analysis. In addition, he worked on microfabricated devices, both in glass and plastics, before joining the start-up company Aclara Biosciences in Mountain View, CA in 1998. Subsequently, he moved to Amersham Biosciences, now GE Healthcare, in Sunnyvale, CA to manage the Advanced Research Team, where he was interested in applying micro-analytical techniques to high throughput applications of genomics and proteomics. In 2003, he joined Bio-Rad to manage the 2D-based proteomics lab. He continued to be interested in all applications of bioanalytical techniques to biological problems.

**Ziad El Rassi** was born in Lebanon (land of Cedars of God http://www.youtube.com/watch?v=NMfvmSdriVM) and received his early education in the school system of Lebanon. The B.S. degree in chemistry was earned at the Lebanese University, Beirut, in 1972, followed by an Education Degree at the same University in 1973. He matriculated to Claude-Bernard University in Lyon, France, for M.S. and Ph.D. degrees in analytical chemistry in 1974 and 1978, respectively. A visiting Assistant Professorship followed at Ecole Centrale de Lyon (Engineering School), France (1978-1980). He then joined the Chemical Engineering Department at Yale University, New Haven, CT, as an Associate Research Scientist (1980-1985) and was appointed to Research Scientist over the period of 1985-1988. An Assistant Professorship was accepted in 1988 at Oklahoma State University (OSU) in the Department of Chemistry. He rose through the ranks to become Full professor in 1998.

El Rassi's current research in separation science is focused on furthering the development of modern liquid phase separation techniques such as high performance liquid chromatography, capillary electrophoresis and capillary electrochromatography by (i) introducing novel separation schemes and principles of high resolving power for biological substances and natural products (ii) investigating the underlying physico-chemical phenomena, (iii) improving the methodology of the three separation techniques, (iv) developing column-based separation platforms for proteomics and (iv) introducing novel applications of general use in the life sciences. http://casb.okstate.edu/testas/chem2010/index.php?option=com_content&task=view&id=39&Itemid=123

During El Rassi's tenure at OSU, he has directed the research of 10 undergraduates, 10 M.S. students, 17 Ph.D. students and 5 Postdoctoral fellows. The research has resulted in 190 papers in peer-reviewed journals, 200 presentations including **140** invited lectures at various locations around the world.

Prof. El Rassi is a member of the permanent scientific committees of The Asia Pacific International Symposium on Microscale Separations, The International Symposium on Capillary Electroseparation Techniques (ITP), and The Latin-American Symposium on Biotechnology, Biomedical, Biopharmaceutical and Industrial Applications of Capillary Electrophoresis and Microchip Technology

Besides serving on the editorial boards of several international journals, Prof. El Rassi is the Editor-In-Chief of the journal ELECTROPHORESIS. He also edited two books on carbohydrate analysis by chromatography and electrophoresis.

Prof. El Rassi has received the following research awards: Sigma Xi Lectureship Award in 2004, Regents Distinguished Research Award in 2005, Oklahoma Scientist of the Year Award in 2006 and Oklahoma Chemist of the Year Award in 2007.

**Pier Giorgio Righetti (**Politecnico di Milano http://www.chem.polimi.it/people/faculty/pier-giorgio-righetti/) has been involved (1970-1980) with the development of isoelectric focusing in soluble, amphoteric buffers. This research has been applied to a number of biomedical and clinical problems, with particular emphasis on the characterization and analysis of genetic mutants of hemoglobin. For about another ten years (1980-1990) he has developed isoelectric focusing in immobilized pH gradients. Already in the early eighties, his group has set-up a novel system for 2-D (charge/mass separations) map analysis, that utilizes as first dimension immobilized pH gradients (IPG). Starting from 1987, he has been involved with the development of novel equipment for large-scale purification of recombinant DNA proteins. The outcome of this project has been a most powerful machine, called multicompartment electrolyzer (MCE) with isoelectric membranes, based on the revolutionary principle of trapping proteins in between sets of amphoteric, buffering membranes. MCE has been used in several projects for extreme purification of proteins, also in view of their crystallization, as well as an enzyme reactor. Recently, the same instrument, miniaturized, has been adopted for pre-fractionation in proteome analysis.

One project he has undertaken during the decade 1990-2000 has been the development of the field of capillary zone electrophoresis (CZE). In CZE, he has worked-out a novel thermal theory, able to predict with accuracy the real temperature inside the capillary during an electrophoretic run. This has allowed the setting of a novel, revolutionary technique: separation of DNA fragments, carrying a point mutation, for the screening of genetic diseases. Separation of PCR-amplified fragments carrying a point mutation cannot be done in isocratic electrophoresis; the mutants can only be resolved in an electrophoretic run in presence of a thermal gradient, along the separation axis. Since it was impossible to obtain in CZE a thermal gradient in space, we have developed thermal gradients in time.

In the latest years, starting from 2000, he has published a series of works, both methodological and applied to biological problems, in proteome analysis. Among the methodological works: a) Use of mass spectrometry for monitoring accidental protein modifications in proteome analysis, linked to the use of current electrophoretic methods on gels; b) Reduction and alkylation of proteins in 2-D maps; c) Modelling of resolution in proteome analysis; d) New statistical approaches in proteome analysis; e) New quantitative approaches in proteome analysis; f) Novel, dilute gel matrices in proteome analysis. Most of his research efforts, in the last few years, have been dedicated to cancer research, along four main lines: a) pancreatic tumours; b) mantle cell lymphomas; c) neuroblastomas; d) mechanisms of chemoresistance of tumour cells to drugs.

**Recognitions**

- Spanish Proteomic Society Award, Segovia, 2011;
- Csaba Horvath Medal award, by the Connecticut Separation Science Council (2008);
- CaSSS (California Separation Science Society) award (October 2006), now in its 12$^{th}$ edition, never before granted to any scientist outside USA;
- Veneto Innovation: first prize for studies on Proteome. 2001;

- Hirai Prize award for outstanding research in Separation Science (Tokyo, Japan). 1999;
- Milano Award: price awarded for advanced genetic research in mutational analysis, offered at the ATB'97 Conference, Milano, November-1997;
- Prize of the English Electrophoresis Society, awarded at the Seattle Meeting of the International Electrophoresis Society, March 1997;
- Prize C.I.B. (Consorzio Italiano di Biotecnologie) for the "Biotechnologist of the year, 1995.

**Lloyd M. Smith** is the W. L. Hubbell Professor of Chemistry and Director of the Genome Center at the University of Wisconsin-Madison, where he has been since 1988. He received an A.B. degree in Biochemistry from the University of California at Berkeley (1977) and a Ph.D. in Biophysics from Stanford University (1981). In 1982 he moved to the California Institute of Technology, where he developed the first fluorescence-based automated DNA sequencing instrument. He has been named one of Science Digest's Top 100 Innovators and has received the Presidential Young Investigator Award, Eli Lilly Analytical Chemistry Award, Association of Biomolecular Resource Facilities Award for the development of automated DNA sequencing, American Chemical Society Award in Chemical Instrumentation, and the Pittcon Analytical Chemistry Award. He has served on the NIH National Human Genome Research Institute Advisory Council, the NIH Human Genome study section, and the NIH Instrumentation and Systems Development study section (chair from 2008-2010). He is an author of over 200 scientific papers and inventor on 24 issued U.S. patents. He has cofounded three biotechnology companies, Third Wave Technologies, GenTel BioSciences, and Apartia Pharmaceuticals. Third Wave was acquired in June 2008 by Hologic, Inc. for $580M. He also served for many years on the Board of Directors of two public companies (Visible Genetics and Third Wave Technologies) and presently serves on the Board of Directors of two private companies, GenTel BioSciences, and Apartia Pharmaceuticals, and as chair of the Scientific Advisory Board for GenTel BioSciences. His primary area of research is in the development of new technologies for the analysis and manipulation of biomolecules.

**Steven A. Soper** received his Ph.D. in Bioanalytical Chemistry from the University of Kansas in 1989 followed by a Postdoctoral Fellowship at Los Alamos National Laboratory, where he worked on single-molecule detection methods for the high speed sequencing of the human genome. Prof. Soper started his academic career at Louisiana State University (LSU) where he held a distinguished Chair in Chemistry. He is currently a member of the Biomedical Engineering and Chemistry Departments at the University of North Carolina, Chapel Hill, NC. He is also the director of an interdisciplinary research center, Center for BioModular Multi-Scale Systems, which was founded in 2004 and was funded by the NSF. Prof. Soper also holds a joint appointment at Ulsan National Institute of Science and Technology in Ulsan, South Korea, where he is a World Class University Professor. His research interests include micro- and nanofabrication of integrated systems for biomedicine, ultra-sensitive fluorescence spectroscopy, high-resolution electrophoresis, sample preparation methods for clinical analyses, and nanofluidics.

Prof. Soper has secured extramural funding from a variety of agencies ($39M USD) and has published over 255 manuscripts in peer-reviewed publications and is the author of six patents. He is also the founder of a startup company, BioFluidica, which is marketing devices for the isolation and enumeration of rare cells from clinical samples. Prof. Soper has supervised 35 PhDs and currently has 12 graduate students working under his direction.

Prof. Soper is working on a variety of projects, including the development of novel tools for molecular analyses, especially in the area of DNA diagnostics for cancers. Specific examples of his projects include; (1) Integrated modular microfluidic systems for the detection of mutations in DNA as a diagnostic for colorectal cancer; (2) Nano-scale systems for the elucidation of the primary structure of DNA and proteins using single-molecule techniques; (3) Analysis of membrane proteins from rare cells; and (4) Selection and enumeration of circulating tumor cells and circulating DNA from clinical samples.

**Recognitions**

- American Chemical Society Award in Chemical Instrumentation (2011)
- Fellow of the AAAS (2010)
- Fellow of the Royal Society of Chemistry (2010)
- Fellow of the Society for Applied Spectroscopy (2010)
- Benedetti-Pinchler Award (2008)
- Distinguished Masters Award at LSU (2008)
- Outstanding Scientist/Engineer of Louisiana (2001)
- R&D 100 Award (1991)
- Associate Editor of the Americas for *Analyst*
- Editorial Board for *Journal of Fluorescence* and *Micro- and Nanosystems*

## Oliver Trapp

Ruprecht-Karls-Universität Heidelberg
Organisch-Chemisches Institut
Im Neuenheimer Feld 270
69120 Heidelberg

1993-1998 Undergraduate and graduate studies in Chemistry at the Eberhard-Karls-University Tübingen, Germany

1998 Diploma in Chemistry and Diploma-Thesis with Prof. Dr. V. Schurig, Institute of Organic Chemistry, Eberhard-Karls-University Tübingen, Germany

1998-2001 Fellow in the DFG Graduate College 'Chemistry in Interphases' at the University of Tübingen, Germany

2001 PhD and Dissertation with Prof. Dr. V. Schurig, Institute of Organic Chemistry, Eberhard-Karls-University Tübingen, Germany

2002-2004 Postdoctoral Fellow in the Group of Prof. Dr. R.N. Zare, Department of Chemistry, Stanford University, California, USA

2004-2008 Assistant Professor at the Max-Planck-Institut für Kohlenforschung in Mülheim an der Ruhr, Germany

2005-2008 Emmy Noether Research Group Leader at the Max-Planck-Institut für Kohlenforschung

Since 10/2008 Full Professor of Organic Chemistry at the University of Heidelberg

Awards

1999-2001 Doctorate scholarship of the Funds of the Chemical Industry (FCI)

2001 Attempto University Prize of the Eberhard-Karls-University Tübingen

2001 Procter&Gamble Innovation Prize

2002 Emmy Noether Postdoctoral Fellowship of the DFG

2003 Prize of the Analytical Chemistry Division of the GDCh

2005 Emmy Noether Research Group of the DFG

2007 Thieme Journal Award

2007 Research Grant Award of the Merck Research Laboratories

2008 Member of the Young College of the Northrhine Westphalian Academy of Sciences

2008 ADUC-Prize of the German Chemical Society (GDCh) for the Best Habilitation Thesis

2008 Heinz Maier-Leibnitz Award of the German Research Foundation (DFG)

2008 Innovation Award 2008 of the Northrhine-Westphalian Ministry of Innovations

2009 Annual Award of the Ruprecht-Karls-Universität Heidelberg for Outstanding Research Achievements

2010 HTC-11 Award, Brugge, Belgium

2010 ERC Starting Grant

**Gyula Vigh** received his undergraduate and doctoral degrees in chemical engineering (1970) and analytical chemistry (1975) from the University of Veszprem, Hungary, and became a faculty in the Department of Analytical Chemistry of the same institution. He immigrated to the USA in 1985 and became a faculty in the Department of Chemistry, Texas A&M University, College Station, TX. He served as Chairman of the Analytical Division at TAMU from 1997 – 2003 and became the inaugural holder of the Gradipore Chair in Separation Science in 2001. He served as one of the editors of Journal of Chromatography and as a member of the editorial boards of the major separation science journals. His current research focuses on the analytical and preparative scale separation of enantiomers and proteins, as well as the synthesis and use of fluorescent derivatizing agents.

**Rui Vitorino** (Department of Chemistry, Materials and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal http://masspec.web.ua.pt/) has been involved in the characterization of saliva using proteomics tools since the 2000. Saliva is a complex biological fluid enriched in glycoproteins and small peptides. As a first approach, salivary proteins were resolved by two-dimensional gel electrophoresis yielding the publication of one of the firsts 2DE maps. Following the main goal of this project, salivary peptides were characterized by LC-MS addressing in few identifications. Later, face to the wide range of different peptides, other approaches were developed which allowed the identification of more than 2000 endogenous peptides in saliva. Nowadays, most of his research has been dedicated to pathologies including dental caries, diabetes and head and neck cancer.
Although his research is addressed to saliva, in the latest years, he has participated in several projects aiming to develop methodologies or find clues through proteome analysis. Among the methodological works: a) organelles fractionation; b) PTM characterization by mass spectrometry; c) protein complex characterization; d) glycoprotein characterization through nanoparticle-lectin immobilized systems.

# Abstracts

# Lectures

## Enantioselective catalysis and analysis in single microfluidic devices

**Detlev Belder**, Institut für Analytische Chemie, Universität Leipzig, 04103 Leipzig, Germany

There is tremendous progress in the miniaturisation of chemical laboratories to the chip-scale either by downsizing chemical reactions or by chip based analytical devices. In order to exploit the ultimate potential of chip-technology in chemistry the different chemical processes should be integrated on a single microfluidic device. Such a microfluidic chip integrating chemical reaction and analyses on a single device is highly attractive, e.g. for high throughput screening and in combinatorial chemistry. In this talk some of the main challenges for the development of an integrated chip based reaction and analysis device are discussed, with a focus on the detection issue (MS and life time fluorescence). Recent results on the integration of on-chip analysis and synthesis for the screening of organo catalysts and bio catalysts are presented.

## Coupling CE to MS: new interfaces, new ion sources new applications

**Christian W. Klampfl,** Institute of Analytical Chemistry, Johannes Kepler-University Linz, Altenbergerstr. 69, A-4040 Linz, Austria

It is more than 30 years since capillary electrophoresis (CE) was coupled to mass spectrometry (MS) for the first time. Since than it has evolved from a quite exotic combination, primarily performed on lab-made equipment, to a well established method within the group of hyphenated techniques [1]. A major driving force for the increased acceptance of CE-MS was the development of suitable interfaces, mainly involving electrospray ionization (ESI), allowing the simple and robust coupling of CE to MS.

Scanning the literature of these last 30 years a series of different interfaces (with respect to their physical design) as well as several different ionization modes have been employed for interfacing CE to MS. These comprise sheathless interfaces, sheath flow interfaces or interfaces including a liquid junction [2]. Among those the co-axial sheath flow interface still plays the most important role. This type of interface is also used in the majority of commercially available devices for CE-

MS. Focusing on ionization, ESI still makes up for almost 99% of all CE-MS applications. Nevertheless alternative approaches employing other ionization techniques have been exploited. These include both, designs using commercially available instrumentation (i.e. adapting HPLC-MS hardware for CE-MS) and also completely lab-made ion sources. A nice example for the latter type is the recently presented atmospheric pressure afterglow ion source for CE-MS [3]. In the present paper different approaches towards the realization of sheath-flow interfaces for CE-ESI-MS are discussed; including investigations using fully commercially available instrumentation as well as interfaces based on commercially available equipment that has been slightly modified and adapted to the specific needs encountered in CE-MS in the laboratory [4]. Thereby the influence of geometry changes (distances and angles) on sensitivity as well as robustness has been tested. The lab-modified interface design provided better results than commercially available devices that often represent a compromise as they often should be usable for both, ultra low flow techniques such as CE and (relatively) high flow separation techniques like HPLC. Regarding ionization, different modes such as ESI, atmospheric pressure chemical ionization, atmospheric pressure photoionization and others are discussed. With respect to parameters like robustness, ease of use and their "unique selling property" regarding the classes of anayltes amenable as well as compatibility of different electroseparation techniques.

**References**


[1] Klampfl CW, Electrophoresis 30 (2009) S83
[2] Schmitt-Kopplin P, Frommberger M, (2003) Electrophoresis 24:3837.
[3] Jecklin MC, Schmid S, Urban PL, Amantonico A, Zenobi R, Electrophoresis 31 (2010) 3597.
[4] S.M. Reiter, W. Buchberger, C.W. Klampfl, Chromatographia 71 (2010) 715.


# CE as tool to investigate the stereodynamics and catalytic reactions


**Oliver Trapp,** Organisch-Chemisches Institut, Ruprecht-Karls-Universität Heidelberg, Im Neuenheimer Feld 270, 69120 Heidelberg, Germany


The investigation of the molecular dynamics of stereoisomers and the study of the kinetics of reactions, in particular of catalyzed reactions, is of fundamental interest in chemistry, biochemistry, and medicine. This presentation focuses on the recent advances to study the stereodynamics of molecules and reaction kinetics of catalyzed processes by means of capillary electrophoresis. Models and algorithms to evaluate interconversion profiles obtained by electrophoretic separation techniques are discussed with respect to the challenging demands of high separation efficiencies typical for electrophoretic techniques. Models used for evaluation are based on iterative computer simulation algorithms using the theoretical plate model or stochastic model of chromatography, empirical calculation methods, and direct access with the approximation function and more recently with the unified equation, which

can be applied to all kinds of first order reactions taking place during a chromatographic or electrophoretic separation. Studies of enantioselective sulfoxidations using immobilized salen ligands in CE will be presented and discussed [6].

**References**

[1] O. Trapp, *Electrophoresis* **2010**, *31*, 786-813.
[2] S. Bremer, O. Trapp, *Electrophoresis* **2009**, *30*, 329-336.
[3] O. Trapp, S.K. Weber, S. Bauch, W. Hofstadt, *Angew. Chem. Int. Ed.* **2007**, *46*, 7307-7310 (Highlighted in *Nature* **2007**, *448*, 730-731).
[4] O. Trapp, *Electrophoresis* **2007**, *28*, 691-696.
[5] O. Trapp, *Anal. Chem.* **2006**, *78*, 189-198.
[6] S. Sandel, S.K. Weber, O. Trapp **2011**, submitted.

## Immuno-monoliths at reduced nonspecific interactions

**Ziad El Rassi,** Gunasena, D.N.
Department of Chemistry, Oklahoma State University, Stillwater, OK 74078-3071, USA

A polar organic polymer monolith was introduced for performing immuno affinity chromatography (IAC) at reduced non-specific interactions. The hydrophilic monolith was prepared by the *in situ* polymerization of glyceryl methacrylate (GMM) as the functional monomer and pentaerythritol triacrylate (PETA) as the crosslinker in the presence of cyclohexanol, dodecanol and water as the porogenic solvent. The polar monolith through its diol groups provides the opportunity to achieve readily the immobilization of antibodies and other affinity ligands for nano liquid affinity chromatography (nano-LAC). In this investigation, anti-haptoglobin antibody was used as the model antibody to study the overall behavior of the immuno-monolith thus obtained in terms of its binding to the antigen (haptoglobin) and to evaluate its non-specific binding with other proteins, especially the high abundant serum proteins such as human serum albumin, transferrin and $\alpha_1$-antitrypsin etc. Due to the presence of hydroxyl groups in the crosslinker and the functional monomer as well, the hydrophilic monolith exhibited negligible non-specific hydrophobic interactions with proteins.

As an extra evaluation of its hydrophilicity, the novel monolith carrying diol functionalities was exploited for its full potentials in normal phase or hydrophilic interaction capillary electrochromatography (HI-CEC). Although the monolith is neutral and void of a fixed charge on the surface, a relatively strong cathodal EOF was observed due to the electric double layer formed by the adsorption of ions from the mobile phase producing a bulk mobile phase flow. The novel monolith can be used to separate polar compounds such as phenol derivatives, small aliphatic amides, nucleic acid bases and nucleosides etc.

## New technologies for the genome age

**Lloyd M. Smith**, Department of Chemistry & Genome Center of Wisconsin
University of Wisconsin-Madison, USA

The successful sequencing of the human genome has provided us with the blueprints of life.... but now the likely greater challenge of understanding those blueprints lies squarely before us. Again, information is needed. The molecules that are encoded by the genome come in many different types and forms. We need to know what molecules are where, when, and in what form, how they change with time and in response to internal and external signals, and how they interact with one another. This information is a first step towards developing an understanding of the complex web of networks and pathways that comprise functioning biological systems. As was true for the Genome Project, new technologies are needed to provide this information. This talk will present challenges, opportunities, and progress in the development of such new technologies, with a particular emphasis on surface science, biological mass spectrometry, and the conjunction of the two.

## Analysis of telomeres and telomerases

**Jiří Fajkus**, *CEITEC MU and Faculty of Science, Masaryk University and Institute of Biophysics ASCR, v.v.i., Brno, Czech Republic*

Biology of telomeres has undergone a widespread development since the initial findings on the molecular nature of chromosome ends and mechanisms of their replenishment by the ribonucleoprotein complex of telomerase, or by alternative pathways. Although the interest in telomeres has been initiated by a pure scientific curiosity, later progress has shown that telomeres and telomerase are intimately connected with the processes of cell proliferation, aging and cancer. In addition, recent papers report on a number of non-telomeric roles of telomerase e.g., in regulation of gene expression or DNA damage response.
In my talk, I would like to explain current approaches to analysis of telomeres and telomerases, together with motivation to perform such analysis, and example results from our research. These will include analyses of telomeric sequences in various groups of organisms, measurement of telomere length, semi-quantitative and quantitative assays for telomerase activity and expression, or evaluation of telomere RNA transcript levels. Moreover, since telomeres are nucleoprotein (chromatin) structures, their functions are mediated and modulated by a number of telomere-associated proteins, and the analysis of DNA-protein and protein-protein interactions thus represents an important input towards understanding telomere functions.

**Acknowledgements**
Our research is supported by the Czech Ministry of Education (MSM0021622415), Czech Science Foundation (P501/11/0289), ASCR (M200040903) and the project "CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from the European Regional Development Fund.

## What is saliva? A perspective of salivary proteomics at University of Aveiro

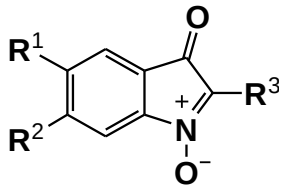
**Rui Vitorino**, Francisco Amado, University of Aveiro, Portugal

Traditionally, saliva is defined as a complex mixture of the secretion of major and minor glandular secretions, in addition to the crevicular fluid, bacteria and epithelial cells. To date, using multiple methods such as polyacrylamide gel electrophoresis (mono and two-dimensional) and liquid chromatography combined with mass spectrometry, over 3000 proteins from different sources have been identified in saliva. Variations in saliva composition have been associated with salivary disorders such as Sjogren's syndrome or dental caries. Saliva, often called "the body mirror", has emerged as an attractive diagnostic fluid facing its simplicity, non-invasive collection and the cost-effective applicability for screening large populations collection. Moreover, it possesses advantageous for various biochemical tests since saliva reflects the concentrations of many blood components. With this presentation, we intend to give a perspective of our approaches and simultaneously present the most relevant findings about salivary proteomics.

## Indolone-*n*-oxides: relation between redox properties and antimalarial activities

**Françoise Nepveu**, University of Toulouse 3, UPS and IRD; UMR 152 PHARMA-*DEV*, 118 route de Narbonne, F-31062 Toulouse cedex 9, France

Although several antimicrobial agents are currently available there is clearly a critical need for the development of new pharmacophores due to the emergence of the resistance of numerous microbes to several drugs. Microbes have a high metabolite rate producing oxidative by-products, which is amplified by the oxidative attack of the host immune system in which reactive oxygen and nitrogen species contribute. The detoxification of reactive oxygen and nitrogen species is a challenge for tissues infected with microbes. Redox metabolism is thus an attractive target for antimicrobial drug development. In the search for anti-infective molecules using their redox properties, so as to disrupt the antioxidant defence systems of the pathogen, we recently show that indolone-*N*-oxide derivatives have antimalarial properties. The pharmacomodulation studies confirmed the central role played by the indolone redox pharmacophore and gave hits with antiplasmodial malarial activities at the nanomolar level. The indolone-N-oxide scaffold is original, was not investigated for its antimalarial potentialities before and gives the possibility to build new chemical series comparatively to the artemisinine-like and chloroquine-like ones, and potentially new drug classes.




Structure of indolone *N*-oxide derivatives

The unique ability of nitrones to form persistent radical adducts and to be involved in oxidation-reduction reactions, lead us to study how the peculiar redox properties of indolone-*N*-oxides might exert a critical action towards the erythrocytic development of *Plasmodium falciparum*, the parasite responsible for malaria. Herein, we report some redox properties of indolone-*N*-oxides in relation with their antimalarial properties.

**References**

[1]Nepveu F., Kim S., Boyer J., Chatriant O., Ibrahim H., Reybier K., Monje MC., Chevalley S., Perio P., Lajoie BH., Bouajila J., Deharo E., Sauvain M., Tahar R., Basco L., Pantaleo A., Turini F., Arese P., Valentin A, Thompson E., Vivas L., Petit S., Nallet J-P., Synthesis and antiplasmodial activity of new indolone *N*-oxide derivatives, *J. Med. Chem*. 2010, 44, 699-714.

## The proteome Argonauts: conquering the “golden fleece” of alcoholic beverages and soft drinks via combinatorial peptide ligands

**Pier Giorgio Righetti**, Elisa Fasoli, Alfonsina D’Amato, Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Via Mancinelli 7, Milano 20131, Italy.

Proteomic science has been vastly exploited in the past ten years for biomarker discovery in sera, in search of panels of proteins able to warn about the onset of various diseases. According to Mitchell (Nature Biotech. 28, 2010, 665-670), this has been the biggest “fiasco” in this arena, with billions of dollars wasted. Completely different results have been obtained by us when analyzing a “fiasco” (a 1.5 liter jug) of white or red wine, with the combinatorial peptide ligand library (CPLL) technology. It turns out that most wine producers treat white wines with casein (and red wines with egg albumen) in order to eliminate residual grape proteins that would flocculate upon long term storage. Although required by EC rulers, no producer has ever stated the residual amount of these allergenic additives in their product. With the CPLL technology, we were able to detect as little as 1 µg casein/L, an extremely high detection sensitivity, unreported up to the present (the official ELISA test of the EC reached barely down to 200 µg/L). However, if untreated wines are analyzed, we can detect well over 100 residual grape proteins present in wines, this suggesting the possibility of proteo-typing grand crus against counterfeited products invading the market. We will additionally report proteo-typing of beers as well as different carbonated soft beverages. One could thus easily distinguish among artificial beverages, made only with synthetic additives and flavours (Coca Cola being a classical example) vs. genuine products made with plant extracts. Regulatory agencies and customers would thus have a new, formidable tool for protection against adulterated and counterfeited foodstuff and beverages.

**References**

Cereda A, Kravchuk AV, D'Amato A, Bachi A, Righetti PG. J. Proteomics 73 (2010) 1732-1739.

D'Amato A, Kravchuk AV, Bachi A, Righetti PG. J. Proteomics 73 (2010) 2370-2377.

Fasoli E, Aldini G, Regazzoni L, Kravchuk AV, Citterio A, Righetti PG. J. Proteome Res. 9 (2010) 5262-5269.
Fasoli E, D'Amato A, Kravchuk AV, Righetti PG. J. Proteome Res. 2011, in press.

## Separation of DNA and DNA-templated metal clusters by CE-LIF

Ming-Feng Huang, Cheng-Kang Chiang, **Huan-Tsung Chang**
Department of Chemistry, National Taiwan University, 1, Sec. 4, Roosevelt Road, Taipei 106, Taiwan

Capillary electrophoresis with laser induced fluorescence (CE-LIF) detection is a powerful technique for the study of DNA conformational changes and characterization of nanomaterials. We have studied the changes in the electrophoretic mobility of the complexes of 5´ end labeled 6-FAM-$T_{33}$ in the presence of $Hg^{2+}$ using CE-LIF. Upon increasing the concentration of $Hg^{2+}$, the electrophoretic mobility for 6-FAM-$T_{33}$ increases, due to its conformation change from a random coil to a folded structure and a decrease in its charge-to-mass ratio. CE-LIF using 2% poly(ethylene oxide) solutions containing OliGreen (fluorophore) and 0.3 mM $Hg^{2+}$ has been applied to the separation of $T_{33}$, $T_5C_{28}$, $T_5C_5T_{23}$, and $T_{15}C_5T_{13}$. We have employed CE-LIF to confirm the purity of fluorescent DNA-templated metal clusters that are interesting and sensitive probes for many analytes such as Cu(II) ions and DNA. Our studies have shown that CE-LIF is useful for the characterization of nanomaterials and can provide information to support the sensing mechanisms of many DNA based probes.

## Capillary electrophoresis analysis of the altered glycosylation of immunoglobulins in autoimmune diseases

**András Guttman** (1), Csaba Váradi (1), Bertalan Meskó (2) and Laszlo Nagy (2)
(1) Horváth Laboratory of Bioseparation Sciences, and (2) Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary

Glycosylation is one of the most important post-translational modifications, which holds the promise to provide numerous biological markers in clinical diagnostics for various diseases. The N-glycosylation on human immunoglobulins, especially on IgG1, plays a critical role in the bioactivity of this group of very important proteins. Significant differences have been reported in IgG1 glycosylation in pregnancy, aging, various autoimmune diseases and multiple myeloma. The four highest abundant glycans of IgG1 are biantennary-agalacto (G0), biantennary-mongalacto (G1 and G1') and biantennary–digalacto (G2) structures. Among these structures, G0 shows the highest variability in the above mentioned normal and pathological conditions. The

aim of this study was to investigate the changes in the relative amount of G0 glycan of IgG1 in such autoimmune diseases as reumatoid arthritis (RA) and Crohn's disease, especially in view of transcriptomics data on galactosyl transferase experession. IgG1 was isolated from human blood samples using Protein A affinity partitioning and the N-glycans were released by peptide-N-glycanase F (PNGase F). The free glycans were then fluorescently labeled with aminopyrene-trisulfonate (APTS) and analyzed by capillary electrophoresis with laser induced fluorescence detection. Glycosylation patterns of sex and age matched samples were compared and analyzed to reveal any possible correlation between IgG1 N-glycan profile, galactosyl transferase expression and the pathogenesis of the disease.

## Microfabricated and Lab-on-a-chip devices in the life science industry: promises, products and problems

**Aran Paulus**, Bio-Rad Laboratories, Laboratory Separation Division, 6000 James Watson Drive, Hercules, CA 94547, USA

Microfabrication on glass and plastic devices set out about 20 years ago to revolutionize analytical workflows in the life science field by making them more automated, with higher throughput through both speed and multiplexing and more integrated by combining multiple steps onto a single chip. Despite some early successes with glass-based chips for RNA, DNA and protein separations, the promised market potential has not yet been reached. What happened and how far are we from finding the killer application for a lab-on-the-chip? This presentation will look at common biological workflows with an emphasis on protein applications and compare traditional techniques such as slab gel electrophoresis and Western blotting with manual sample processing steps to chip-based approaches. The comparison will include a look at the practical aspects of the complete workflows, critical time-dependent steps and analyte concentration and absolute amounts for each step in the protocol.

## Single-molecule electrochromatography in nano-columns with conductivity readout: a novel approach for high throughput DNA sequencing

**Steven A. Soper**,[1,3] Sunggook Park,[2] Dimitris Nikitopoulos,[2] Yoonkyoung Cho,[3] Heungjoo Shin,[3] Dorel Moldovan,[2] Franklin Uba,[1] Dong-Kyu Park,[3] Jiahao Wu[2] and Mateusz Hupert[1]
[1]Department of Biomedical Engineering, Department of Chemistry, University of North Carolina, Chapel Hill 27599, USA
[2]Department of Chemistry and Department of Mechanical Engineering, Louisiana State University, Baton Rouge, LA 70803, USA
[3]Ulsan National Institute of Science and Technology (UNIST), Ulsan, South Korea

Complete integration of the sample processing pipeline into a single fluidic system is of interest for potential point-of-care applications, especially in the area of *in vitro*

diagnostics. Microfluidic systems are a promising technology platform as they can provide automated sample handling and reagent delivery as well as timely results with minimal operator intervention. While microfluidics offers some compelling advantages for molecular processing, systems using *nanofluidics* offers new opportunities that are not accessible via microfluidics. In particular, unique physics occur at the nanometer scale. In this presentation, we will discuss the development of a nanofluidic system for biopolymer analysis, such as DNAs, RNAs and proteins. The microfluidic components used for sample preparation prior to entering the nanofluidic system, were made from conventional thermoplastics using high production rate replication technologies, such as injection molding. The microfluidic system could be interfaced to a nanofluidic system fabricated via nano-replication technologies, such as nanoimprint lithography (NIL). One of the nanofluidic elements consisted of a column with dimensions on the order of 50 x 50 nm (depth x width) with a length of 60 µm. With the addition of an applied longitudinal electric field, single molecules could travel through this nano-column experiencing wall interactions predicated on the nature of the molecule and the surface structure of the column (nano-capillary electrochromatography). Thus, single molecules could be identified through their unique mobility through the nano-column. We will discuss the ability to fabricate nano-scale molds in Si using focused ion beam milling with the masters used to create polymer stamps for the high scale production of these nano-scale chromatography columns. Molecular Dynamic (MD) simulation results have been secured as well to demonstrate proof-of-concept for moving single molecules through nano-columns with the migration time used for their molecular identification.

## Microfluidics for applications in chemistry

**Andreas Manz**, Jörg Ingo Baumbach, Pavel Neuzil
KIST Europe, Campus E71, 66123 Saarbrücken, Germany

Microfluidics and miniaturization of detectors are important components for future clinical diagnostics instrumentation. The field has developed now for many years, and has its niche products in genomics, proteomics and in combination with sensors. Initially, the driving forces were curiosity and the drug discovery application. However, interests have shifted towards clinical applications in recent years.
Several aspects will be highlighted, like non-invasive diagnostics applications in breath analysis for infectious diseases, "generic" product development in the electrophoresis & polymerase chain reaction area, and novel approaches to micro/nano multiphase systems using droplet generators for pseudo-crystalline emulsions.

# Isoelectric focusing coupled to mass spectrometry for bioanalysis

**Kilár Ferenc**[1,2], Páger Cs[1], Dörnyei Á[1], Vargová A[2], Takácsi-Nagy A[1], Thormann W[3]
[1]Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12, H-7624 Pécs, Hungary
[2]Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary
[3]Department of Clinical Pharmacology and Visceral Research, University of Bern, Murtenstrasse 35, CH-3010 Bern, Switzerland

Isoelectric focusing is one of the most important separation techniques in proteomics. Its application is inevitable in obtaining information on the appearance, as well as up and down regulation of proteins determined by the commonly used 2D polyacrylamide gel-electrophoresis and combined with off-line mass-spectrometry. The introduction of capillary isoelectric focusing leads, however, to a new opportunity in those analytical studies. An on-line combination of CIEF with an appropriate mass spectrometric detector, is able to replace 2D-PAGE, with high throughput and a much less laborious manner. The coupling of isoelectric focusing with mass spectrometry is, however, still a challenge in bioanalysis. The lecture will summarize the steps and future opportunities in this topic and discuss the various methodological aspects. CIEF in presence of electroosmosis with sequential injection of carrier ampholytes and sample was found to be suitable for mass spectrometry detection. The separate injection of the sample and the ampholytes provides good condition to suppress and overcome the undesirable effect of the presence of ampholytes in mass spectrometry. By the appropriate selection of ampholyte solutions, whose pH range not necessarily covers the p*I* values of the analytes, the migration of the components can be controlled, and the impact of the ampholytes on mass spectrometric detection is decreased. The unique applicability of this setup is shown by testing several parameters, such as, the application of volatile electrolyte solutions, the type of the ampholytes, the order and the number of the ampholyte and sample zones. Broad and narrow pH range ampholytes were applied in experiments using uncoated capillaries with different length for the analyses of substituted nitrophenol dyes to achieve optimal conditions for the mass spectrometric detection [1]. Although, the sample components are not leaving the pH gradient, due to the decrease in the ampholyte concentration at the position of the components, and because the sample components migrate in charged state, the ionization is more effective for mass spectrometry detection. Results with model calculations confirm the mechanism of the process in the isoelectric focusing using one or two ampholyte zones before and/or after the sample zone [2].

**Acknowledgements**
This work was supported by the grants SROP-4.2.2/08/1/2008-0011 and TÁMOP-4.2.1. B-10/2/KONV-2010-0002, and by the Swiss NSF.
References: 1. Páger, C. et al. (2011) Electrophoresis 32, 1875-1884.
2. Takácsi-Nagy, A. et al. Electrophoresis (submitted for publication)

# Design, synthesis and analytical characterization of a new family of pI markers

**Gyula Vigh,** Department of Chemistry, Texas A&M University, College Station, TX 77842-3012, USA

In continuation of our efforts to introduce fluorescent derivatizing agents optimized for the various operation modes of capillary electrophoresis, we have designed a new family of structurally and spectrally homogeneous pI markers for use in cIEF, and synthesized and analytically characterized the first seven members of the family. The new pI markers can be detected by both absorbance and fluorescence detectors. Their $\lambda^{excitation}_{max}$ is at 495 nm, $\lambda^{emission}_{max}$ is at 525 nm. The excitation spectra are broad enough for excitation with any laser in the $440 < \lambda^{excitation} < 510$ nm range. Their fluorescence spectra do not change when the pH of their solution is varied in the $2.2 <$ pH $< 11$ range, or when the buffering species in the background electrolytes are changed or when the pI markers are dissolved in different narrow pI range carrier ampholyte fractions ($3 <$ pI $< 4$; $4 <$ pI $< 6$; $5 <$ pI $< 8$ and $8 <$ pI $< 10$).

Since the new pI markers contain multiple protic groups with closely spaced $pK_a$ values, their tentative pI values were characterized by both capillary zone electrophoresis in a series of background electrolytes whose pH values bracketed the pI values of the markers as described in Ref. 1, and by cIEF using broad-range carrier ampholytes, UV-absorbing primary pI markes (ElphoMarks) and pressure mobilization. The tentative pI values of the first seven members of the new family are 3.07; 4.16, 5.74; 7.45; 8.34; 9.42 and 10.50, respectively.

**References**

[1] P. Glukhovskiy, G. Vigh, *Electrophoresis,* **19**, 3166- 3170 (1998).

# Investigation of biopeptide interactions by capillary electrophoresis

**Kašička Václav**, Ehala S., Šolínová V., Schimperková T., Sázelová P., Koval D.
Institute of Organic Chemistry and Biochemistry, v. v. i., Academy of Sciences of the Czech Republic, Flemingovo 2, 166 10 Prague 6, Czech Republic

Identification and quantification of biopeptide interactions with other (bio)molecules is of extraordinary importance since most of peptide biological functions are implemented *via* these interactions. The strength of these interactions is quantitatively characterized by the binding (stability, association) constant, $K_b$, of peptide complexes. Among the methods employed for $K_b$ determination, affinity capillary electrophoresis (ACE) possesses several advantages – small sample amount (typically picomole of analyte in nanoliter applied volumes), simultaneous $K_b$ determination of complexes of different analytes, the analytes need not be quite pure and their concentration need not be exactly known [1, 2].

In this contribution, application of ACE to investigation of non-covalent interactions of valinomycin, macrocyclic dodecadepsipeptide antibiotic ionophore, with ammonium and alkali metal ions in methanol will be presented. The apparent binding constants of the valinomycin-cation complexes were obtained from the ACE measurements of the dependence of valinomycin effective mobility on the cation concentration in the background electrolyte (BGE) using a non-linear regression analysis. The determined $K_b$ values of the above complexes confirmed a considerably higher selectivity of valinomycin for $Rb^+$, $K^+$ and $Cs^+$ ions (with log $K_b$ in the range 4.63 – 3.81) over $NH_4^+$, $Li^+$ and $Na^+$ ions with log $K_b$ between 1.45 and 1.54 [3, 4]. In addition, binding constants of the complexes of enantiomers of an antimicrobial dipeptide H-β-Ala-D,L-Tyr-OH and its derivatives (*N*-Ac-β-Ala-D,L-Tyr-OH, and H-β-Ala-D,L-Tyr-$NH_2$) with a chiral selector, 2-hydroxypropyl-β-cyclodextrin, have been evaluated by nonlinear fitting of the mobility data to regression model describing the dependence of the effective mobilities of these peptides on cyclodextrin concentration in the BGE. Prior to $K_b$ calculation, the effective mobilities have been corrected to reference temperature and constant ionic strength and constant viscosity of the applied BGEs. ACE proved to be a suitable method for investigation of both weak and medium-strong interactions of charged as well as non-charged biopeptides with ligands of different character and size.

**References**

[1] Chen, Z., Weber, S. G. *Trends Anal. Chem.* 2008, *27/9*, 738-748.
[2] Winzor, D. J. *Anal. Biochem.* 2008, *383/1*, 1-17.
[3] Ehala, S., Kašička, V., Makrlík, E. *Electrophoresis* 2008, *29*, 652-657.
[4] Ehala, S., Dybal, J., Makrlík, E., Kašička, V.: *Electrophoresis* 2009, *30*, 883-889.

**Acknowledgements**

The work was supported by the Czech Science Foundation, grants nos. 203/08/1428 and 203/09/0675, and by the Research Project AV0Z40550506 of the Acad. Sci. of CR.

# Posters

## 1
## Investigation of matrix deposition techniques for high throughput MALDI mass spectrometry imaging

Bednařík A.[a], Ertl L.[a], Rážová I.[a], Tomalová I.[a], Moskovets E.[b], Preisler J.[a]
a) Department of Chemistry, Faculty of Science and Central European Institute of Technology, CEITEC, Masaryk University, Brno, Czech Republic
b) MassTech, Inc. 6992 Columbia Gateway Drive, Suite #160, Columbia, MD 21046, USA

Matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is an emerging technique with an immense biochemical and pharmaceutical potential, which possesses ability to show spatial distribution of specific compounds in tissues. Here, we will present a comparison of common MALDI-MSI sample preparation techniques. The MALDI matrix together with rhodamine B labeled angiotensin I was deposited on sample MALDI plate by three different approaches, namely by electrospray, spin-coating and piezoelectric nano-spotter. The homogeneity of deposited samples was studied under microscope either by observing simple optical image or in the case of piezo-deposited nano-spots by observing fluorescence image after irradiating the sample with 532 nm laser. Optical image was compared with the MSI generated map of peptide distribution along the samples. To measure spatial distribution of selected peptides, a laboratory-built high-throughput axial time-of-flight (TOF) mass spectrometer with 2 kHz 355 nm Nd:YAG laser was used.

**Acknowledgements**
This work was supported by The Czech Science Foundation (P206/10/J012), by The Ministry of Education, Youth and Sports (MSM0021622415) and the project "CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund.

# 2
# Stability constants of charged cyclodextrins with neutral analytes - determination by CE

Beneš M., Svobodová J., Zusková I., Gaš B.
Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43, Prague 2, Czech Republic
E-mail: martinxb1@seznam.cz

Stability constant characterizes binding interaction between an analyte and complexation agent. These interactions play very important role in separation processes of, in other way undistinguishable, compounds, e.g. enantiomers. The most widely used complexation agents are cyclodextrins (CD). Charged cyclodextrins significantly extend the applicability of the electrophoretic methods for neutral analytes.

Affinity capillary electrophoresis (ACE) belongs to methods suitable for the determination of stability constants[(0,0)]. If charged CDs are used, the attention must be paid not only to viscosity of the BGE and to the influence of Joule heating on the temperature in the capillary but also to the increasing ionic strength.
The thermodynamic stability constants of R- and S-hydrobenzoin and R- and S-(3-brom-2-methyl-1-propanol) with cationic modified β-cyclodextrin: 6-monodeoxy-6-mono(3-hydroxy)propylamino-β-cyclodextrin hydrochlorid (PA-β-CD) were determined by ACE. The average temperature (25°C) of the BGE in the capillary was kept constant. This was achieved by decreasing of the cassette temperature (based on the conductivity measurements). The viscosity correction was performed using the viscosity ratio[(3)]. The increase of ionic strength due to increasing PA-β-CD concentration in the BGE was compensated by changing of the concentration of the separation buffer. The nonlinear regression was used to analyze of the experimental data. In the next step, the dependence of the analyte effective mobilities on PA-β-CD concentration was measured without the ionic strength compensation to demonstrate the influence of ionic strength on the stability constant. A new procedure was established to estimate stability constants from such data, too. These stability constants are in very good agreement with those obtained at the constant ionic strength.

**References**

1. Ušelová-Včeláková K., Zusková I., Gaš B. *Electrophoresis* 2007, *28*, 2145-2152
2. Tanaka Y., Terabe S. *J. Chromatogr. B* 2002, *768*, 81-92
3. Bowser M. T., Sternberg E. D., Chen D. D. Y. *Electrophoresis* 1997, *18*, 82-91

**Acknowledgements**

The support of the Grant Agency of the Czech Republic, grant no. P205/11/J043, Grant Agency of Charles University, grant no. 323611, CEEPUS network, the long-term research plan of the Ministry of Education of the Czech Republic (MSM0021620857) and Cyclolab (Budapest, Hungary) for donation of the cyclodextrin PA-β-CD are gratefully acknowledged.

# 3
# The use of intrinsic time-resolved fluorescence detection in miniaturized analysis systems

Beyreiss, R., Ohla, S., Nagl, S., Belder D.
University of Leipzig, Institute of Analytical Chemistry, Weissenfelser Str. 67, 04229 Leipzig, Germany

Lab-on-chip technology is an emerging and fast growing research field applicable in many life science disciplines. The miniaturization and transfer of chemical procedures into multifunctional microfluidic devices leads to intensification and acceleration of the whole process. Due to the small microscale dimensions, miniaturized systems demand highly sensitive detection techniques. In this respect, one commonly used technique is fluorescence detection, which however requires suitable fluorophores. Introducing extrinsic chromophores can be troublesome. One possibility to circumvent labeling procedures is to shift the excitation wavelength in the deep UV spectral region and thus inducing intrinsic fluorescence of various analytes [1, 2].

Additional molecular information about the analytes can be gathered by applying time-resolved fluorescence detection [3, 4]. Since the parameter lifetime is a molecular signature, lifetime determination enables peak assignment and distinction between different analytes. Another feature of time-resolved detection is the possibility to discriminate background contributions, resulting in enhancement of sensitivity, which is especially crucial in miniaturized systems.

In this context, we established intrinsic, time-resolved fluorescence detection in microchip electrophoresis. A mixture of the small aromatics serotonin, propranolol, 3-phenoxy-1,2-propanediol and tryptophan was used for system validation [4]. In the experimental setup a 20 MHz picosecond laser operating at 266 nm served as excitation source and emitted fluorescence was detected using time-correlated single photon counting (TCSPC). Furthermore, the detection technique in lab-on-a-chip applications was demonstrated by investigation of on-chip Pictet-Spengler conversion. The conversion of dopamine by acetaldehyde was carried out within an integrated serpentine micromixer. Subsequently the residual substrate and the enantiomeric product salsolinol were separated on the same microchip and detected online by TCSPC. Fluorescence lifetime determination of the separated compounds allowed peak identification [5].

**References**

[1] Li, Q., Seeger, S. *Appl. Spec. Rev.* 2010, *45*, 12–43.
[2] Suhling, K.; French, P. M. W.; Phillips, D. *Photochem. Photobiol. Sci.* 2005, *4*, 13–22.
[3] Llopis, S. D.; Stryjewski, W.; Soper, S. A. *Electrophoresis* 2004, *25*, 3810–3819.
[4] Beyreiss, R., Ohla, S., Nagl, S., Belder, D. *Electrophoresis* 2011, *accepted.*
[5] Ohla, S. ,Beyreiss, R., Fritzsche, S., Gläser, P., Nagl, S., Stockhausen, K., Schneider, C., Belder, D. *Chem. Eur. J.* 2011, *accepted.*

# 4
# Polyethyleneimine coated capillary in nucleotide and short oligonucleotide analysis

Bittová M.[1], Roblová V.[1], Majerová, E.[2], Trnková L.[1]
[1] Department of Chemistry, Faculty of Science, Masaryk University, Kotlarska 2, 611 33 Brno, Czech Republic.
[2] Department of Experimental Biology, Faculty of Science, Masaryk University, Kotlarska 2, 611 37 Brno, Czech Republic.

In last few decades, there have been increasing demands for fast and simple analysis of nucleotides and short oligonucleotides from biological matrices. Their separation and purification is fundamental in pharmaceutical and clinical studies as well as in new biotechnology development.

Capillary zone electrophoresis as a powerful analytical technique provides a versatile tool for nucleotide and oligonucleotide analysis. Inner surface modification of fused-silica capillaries with appropriate chemical reagents along with electroosmotic flow control allow targeted selection of separation system for analytes of interest. Polyethyleneimine (PEI) is polycationic highly branched polymer with positively charged primary, secondary and tertiary amino groups in the structure that can be used as fused-silica capillary modifier. It was firstly introduced by Towns and Regnier [1]. Its irreversible adsorption on the silanol groups of the fused-silica capillary surface eliminates negative charge and excess of charged amino groups cause reversion of electroosmotic flow towards the anode. However, consequential interactions with background electrolyte can alter the charge again, thus the electrolyte for analysis must be chosen carefully [2].

In our work, we successfully applied polyethyleneimine coated capillaries in analysis of nucleotides and synthetic pentamers. Also, we studied the influence of particular component of Tris/boric acid/EDTA buffer (TBE) on the PEI coated capillary surface [4]. Furthermore, the effect of hydroxyethylcellulose (HEC) and/or β-cyclodextrin on the nucleotide separation was followed up. Finally, we analyzed restricted DNA from *Nicotiana tabacum* and the representation of nucleotides was evaluated.

**References**

[1] Towns, J., K., Regnier, F., E., *J Chromatogr A* 1990, *516*, 69-78.
[2] Spanila, M., Pazourek, J., Havel, J., *J Sep Sci* 2006, *29*, 2234-2240.
[3] Nutku, S., M., Erim Berker, F., B. *Turk J Chem* 2003, *27*, 9-17.
[4] Wang, P., Ren, J. *J Pharmaceut Biomed* 2004, *34*, 227-283.

**Acknowledgements**

This work was supported by a project of Ministry of Education, Youth and Sports of the Czech Republic - LC06035.

# 5
# On-line two-dimensional liquid chromatography-capillary electrophoresis separations of phenolic compounds

Česla P., Dinisová P., Fischer J., Jandera P.
University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, Studentská 95, CZ-53210 Pardubice, Czech Republic

Separation of complex samples containing analytes with wide range of physicochemical properties is often difficult in one-dimensional separation system. The resolution can be significantly improved by subjecting the effluent outcomingfrom first separation system to the second separation based on different principle. The highest improvement of resolving power is usually achieved when the separation principles used in both dimensions are completely independent such as combination of liquid chromatography and capillary electrophoresis. The simpler off-line approach is not strictly limited by the volume of transferred fractions and differences in geometry of LC column and CE capillary [1]. On-line connection offers shorter analysis time and higher sample throughput, but it requires special instrument modification or even construction of new separation system [2,3].

In this work, new approach to on-line two-dimensional separation method combining capillary liquid chromatography with capillary electrophoresis is presented [3]. Capillary columns with fused core C8 and C18 silica particles were applied in the first separation dimension. In the second dimension, the short capillaries with extended light path cells were applied for fast separation of compounds in fractions transferredfrom LC separation. The transfer of the fractions between the two dimensions is based on the application of electronically controlled switching valve. The separation conditions in both LC and CE dimensions were optimized using a set of representative standards of phenolic compounds. Further, optimized programmed separation conditions in both dimensions were used to improve the sample resolution and peak capacity in comparison to the isocratic system. The on-line two-dimensional method under optimal conditions was applied on the separation of phenolic compounds in environmental and food samples.

**References**

[1] Česla, P., Fischer, J., Jandera, P. Electrophoresis 2010, 31, 2200-2210.
[2] Bushy, M.M., Jorgenson, J.W.Anal. Chem. 1990, 62, 978-984.
[3]Česla, P., Dinisová, P., Fischer, J., Jandera, P. Chromatographia, submitted.

**Acknowledgements**

The authors acknowledge the financial support by the Czech Science Foundation, project No. 203/09/P199.

# 6
# Development of a validated capillary electrophoresis method for enantiomeric purity control and quality control of levocetirizine in a pharmaceutical formulation

Deng X.[*], De Cock B., Adams E., Van Schepdael A.
Katholieke Universiteit Leuven, Faculteit Farmaceutische Wetenschappen, Laboratorium voor Farmaceutische Analyse, O&N2, PB 923, Herestraat 49, B-3000 Leuven, Belgium

Levocetirizine, a single-enantiomer drug, is the active enantiomer of the second generation antihistaminic drug cetirizine. It is an oral histamine $H_1$ receptor antagonist of the latest generation that is licensed for the symptomatic treatment of allergic rhinitis, with twice the affinity for the $H_1$ receptor compared with cetirizine and is a potent antihistamine as demonstrated by inhibition of histamine-induced weal and flare reactions and in clinical studies.

A chiral capillary electrophoresis method has been developed for the quantification of 0.1 % of the enantiomeric impurity (dextrocetirizine) in levocetirizine and determination of both in pharmaceuticals using sulfated-*β*-cyclodextrins as chiral selector. Several parameters affecting the separation were studied such as the type and concentration of chiral selectors, buffer composition and pH, organic modifier, mixtures of two cyclodextrins in a dual system, voltage and temperature. The optimal separation conditions were obtained using a 50 mM tetraborate buffer (pH 8.2) containing 1 % (w/v) sulfated-*β*-cyclodextrins on a fused-silica capillary. Under these conditions, the resolution of racemic cetirizine was higher than 3 and the chiral impurity migrates in front of levocetirizine, which is suitable to determine enantiomeric impurity. In order to validate the method, the stability of the solutions, robustness (two level half fraction factorial design for 5 factors using 19 experiments ($2^{n-1}+3$)), precision of the method, linearity (dextrocetirizine 0.25-2.5 µg/ml, $R^2$=0.9994 y=0.0375x+0.0008; levocetirizine 15-100 µg/ml, $R^2$ =0.9996 y=0.0213x+0.0339), limit of detection (0.075 µg/ml, 0.03 % m/m), limit of quantification (0.25 µg/ml, 0.1 % m/m), accuracy (dextrocetirizine 84-109 %, levocetirizine 97.3-103.1 %), filter effect and different CD batches were examined. The validated method was further applied to bulk drug and tablets of levocetirizine.

# 7
# Characterization of lipid A acylation patterns in rough-type bacterial strains using MALDI-TOF MS in reflectron mode

Dörnyei Á.[1,*], Kilár A.[2], Sándor V.[2], Kocsis B.[3], Kilár F.[1,2]
[1] Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12, H-7624 Pécs, Hungary
[2] Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary
[3] Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Pécs, Szigeti út 12, H-7624 Pécs, Hungary

Most Gram-negative bacteria have lipopolysaccharide (LPS, also called endotoxin) on their outer membrane. In general a LPS consists of a phosphoglycolipid part (termed lipid A) and two variable oligosaccharide (OS) regions (the core OS and the O-specific polysaccharide), meanwhile it is the lipid A domain that is held responsible for the endotoxic activity. The rough (R)-type LPSs do not possess any O-specific polysaccharide and sometimes lack portions of the core, as well. Lipid A has mostly phosphorylated β-D-Glc*p*N-(1'→6)-α-D-Glc*p*N (Glc*p*N: 2-amino-2-deoxy-glucopyranose) disaccharide backbone decorated with multiple fatty acid side chains. The acylation and phosphorylation patterns strongly influence the charge state and secondary structure of lipid A and consequently, its endotoxic activity.

Lipid A samples are generally prepared from isolated LPS samples with mild acidic hydrolysis to split the acid-labile ketosidic linkage between the lipid A and core OS parts. The disadvantages of this chemical degradation step are the high risk of losing acid-labile, but biologically important, functional groups and the enhancement of the intrinsic heterogeneity of the product. Distinguishing laboratory artifacts from natural heterogeneity is one of the major analytical challenges when dealing with LPS and lipid A preparations.

In this study, we show that the negative-ion, reflectron mode MALDI-TOF MS technique can be used to identify the lipid A components with different acylation patterns directly from R-type LPS samples without performing the chemical isolation of lipid A prior to the mass spectrometric measurement. Four R-type *Shigella sonnei* strains (*S. sonnei* 4350, R41, 562H, and 4303) with different core OS parts were used to demonstrate the usefulness of this method.

**Acknowledgement**
Á.D. acknowledges the support of the János Bolyai Research Scholarship (Hungarian Academy of Sciences).

# 8
# Divergent flow isoelectrofocusing of whey caseinomacropeptide

Duša F.[1,2], Moravcová D.[2], Kahle V.[2], Šlais K.[2]
[1]Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno,Czech Republic
[2]Department of liquid phase separations,Institute of Analytical Chemistry AS CR, v. v. i.,Veveří 97, 60200Brno, Czech Republic

Isoelectric focusing (IEF) is an efficient method for separation of macromolecules. Classic IEF formats as capillary or gel are not suited for a processing of high volume of a sample. This disadvantage is solved by free flow IEF. Thanks to continuous flow of sample and ongoing separation the technique could be conveniently used for pre-separation and possibly separation of analytes. Method of divergent flow IEF (DF-IEF) invented in our department [1]further improves separation efficiency of currentfree flow IEF instrument pattern. Based on our previous research [2-4] we used the DF-IEF for concentration and purification of caseinomacropeptide (CMP). CMP originates during cheese making process by cleavage ofκ-casein with chymosin. It is heterogeneous group of differently glycated polypeptides based on the same primary amino acid structure. We used sweet bovine whey as a source of CMP since it composesabout 25 % of whole whey protein. Particular focused fractions originated from sweet bovine whey were analyzed by capillary HPLC with C18 reverse phasecolumn and UV detection.Presence of CMP in fractions was confirmed by comparison of obtained chromatograms with results of HPLC analysis of CMP standard. Results showed an ability of device to separate CMP from α-lactalbumin and β-lactoglobulin. We proved that DF-IEF may be used for CMP purification as atechniquealternativeto ultrafiltration and ion-exchange chromatography.

**References**

[1] Slais, K., *Electrophoresis* 2008, *29*, 2451-2457.
[2] Stastna, M., Slais, K., *Electrophoresis* 2008, *29*, 4503-4507.
[3] Mazanec, K., Bobalova, J., Slais, K., *Analytical and Bioanalytical Chemistry* 2009, *393*, 1769-1778.;
[4] Stastna, M., Slais, K., *Electrophoresis* 2010, *31*, 433-439.

**Acknowledgements**

This work was supported by the Grant Agency of the Academy of Sciences of the Czech Republic No. IAAX00310701 and by the Institutional research plan AVO Z40310501. The financial support of the Ministry of Education, Youth and Sports of the Czech Republic by grant No. LC06023 and by the Grant of Ministry of Interior No. VG20102015023is also gratefully acknowledged.

# 9
# Laser diode thermal vaporization inductively-coupled plasma mass spectrometry

Foltynová P.[b], Kanický V.[b], Preisler J.[a,b]
[a]Central European Institute of Technology, CEITEC MU, Masaryk University, Brno, Czech Republic, preisler@chemi.muni.cz, Kamenice 5, 625 00 Brno, Czech Republic
[b]Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic

A new approach of sample introduction to inductively coupled plasma mass spectrometry (ICP-MS), laser diode thermal vaporization (LDTV), is presented. The method does not require an expensive high-energy pulse laser, because the technique employs a low-cost NIR diode laser which makes it a promising alternative to nebulizers. Defined submicroliter volumes of samples are deposited on a suitable sample carrier with an absorber, such as common office paper with commercial printer black ink. The diode laser energy is sufficient to induce burning and/or pyrolysis of the carrier substrate sample which leads to aerosol generation. Selection of optimal conditions, e.g., experimental arrangement, raster, scan speed, additives etc. will be discussed. The limits of detection of Co, Ni, Zn, Mo, Cd, Sn and Pb deposited on the preprinted paper were found to be in the range of 4 – 300 pg. Applicability was demonstrated on determination of lead in whole blood and tin in canned food. Using a multielemental calibration set prearranged on the carrier, LDTV ICP MS provided rapid and reproducible (10%) quantitative analysis of metals. The advantages of LDTV are also easy sample archiving, very low consumption of sample solution, transportation and simplicity of sample preparation.

**Acknowledgements**
Authors acknowledge support of the Czech Science Foundation (Grant No. 203/09/1025), the Ministry of Education, Youth and Sports of the Czech Republic (MSM0021622411, MSM0021622412 and MSM0021622415) and by the project "CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund.

# 10
# Effective pre-concentration and analysis of heavy metals by use of ligand step gradient focusing

Glovinová, E., Pospíchal J.
Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic, posp@mendelu.cz

The capillary electrophoretic method for the focusing and selective pre-concentration of metal chelates with subsequent on-line ITP analysis was developed and verified. The selected ions of heavy metals ($Pb^{2+}$, $Cd^{2+}$) were pre-concentrated from the mixture and analyzed. Focusing of metals was carried out in ligand field step gradient, which was created by an addition of a convenient ligand agent to the regular stationary pH step gradient.

During the first step, the metal ions were continuously dosed into the column, where they were selectively trapped on the stationary ligand step gradient in the form of non-moving zones of citrate complexes with effective zero charge. After accumulation of detectable amount of analyte, the dosing was stopped and accumulated zones were mobilized to the analytical column, where they were analyzed by ITP method with conductivity or photometric detection. The proper electrolyte systems for the dosing (mode IEF), mobilizing (mode MBE) and analytical step (mode ITP) were developed and tested.

For the focusing and ITP analysis, the following electrolytes were used:
Alcaline primary (focusing) electrolyte: LE/PE: 0.01M $NH_4Ac$ + 0.01M $NH_4OH$ + 0.002M ammonium hydrogencitrate + 1% polyethylenglycole, pH=9.24.
Acidic dosing (focusing) electrolyte: DE: 0.01M HAc + 0.01M $NH_4Ac$ + 1% tritone X100, + sample metals. pH=4.75.

It was found out from the experiments outcomes, that we reached practical limits of the LSGF method, nearly $10^3$- fold accumulation, from the concentration terms a cca $10^6$- fold increase in the concentration, which is similar to limits reached by CAF-IEF.

A continuous dosing technique was used for the lowering of detection limit, with the dosing time of 2000-3000 sec. cLOD can be lowered by 739/686 fold for a Cd and Pb respectivelly. For example, analysis of tape water is given, with results $1.0x10^{-8}$M $Cd^{2+}$ and $0.9x10^{-8}$M $Pb^{2+}$; such a sample cannot by analysed by routine ITP analysis.

**Acknowledgements**
This work was supported by the Grant Agency of the Czech Republic, Grant No. 206/10/1219.

# 11
# Microfluidic liquid junction system for CE/MS

Grym J.,Křenková J., Foret F.
Institute of Analytical Chemistry ASCR v. v. i., Brno, Czech Republic

Mass spectrometry (MS) represents one of the most important tools for bioanalysis. At present electrospray is the dominant method for on-line coupling of microscale separations with MS. Commercially available MS instrumentation is, in many respects, mature and provides robust MS coupling with chromatographic separations. On the other hand capillary electrophoresis/MS coupling is still an evolving technology. In this work we have designed experimental systems suitable for electrospray coupling of microcolumn separations and especially capillary electrophoresis. This system is based on hybrid microfluidics design where the microfabricated part serves as a manifold for attachment of the separation capillary and connection of the electrode reservoirs. The device is designed as a hybrid capillary/microfluidics manifold system. The microfabricated part incorporates self-aligning liquid junction for precise positioning of the CE separation capillary and fused silica electrospray needle and connections for the external electrode reservoirs. The interface is constructed as an external part attached to the sampling orifice of the mass spectrometer via a miniature subatmospheric electrospray chamber. The system performance was characterized with respect to the signal intensity and practical use for analyses of peptides, proteins and oligosaccharides.

# 12
# Steady-state and time-resolved fluorimetric approaches in the study of interactions of isoquinoline alkaloids with biomacromolecules

Cheng P.[a], Zatloukalová M.[b], Vacek J.[b], Zeng J.-G.[a], Šimánek V.[b], Kubala M.[c], Ulrichová J.[b]

[a]) Hunan Agricultural University, Changsha 410128, People's Republic of China, [b]) Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, 775 15 Olomouc, Czech Republic, [c]) Department of Biophysics, Faculty of Science, Palacký University, 17. listopadu 12, 771 46 Olomouc, Czech Republic

Steady-state and time-resolved fluorescence spectroscopy methods were used for the study of bioactive isoquinoline alkaloids (sanguinarine, chelerythrine, protopine and allocryptopine, see Fig. 1). The fluorescence approaches were applied for examination of interconversion between positively charged cationic form and free base form of the alkaloids in dependence on pH. Using optimized experimental protocols the interactions of the alkaloids with selected biomacromolecules as proteins ($Na^+/K^+$-ATPase and aromatic L-amino acid decarboxylase) and various structural forms of DNA were described.

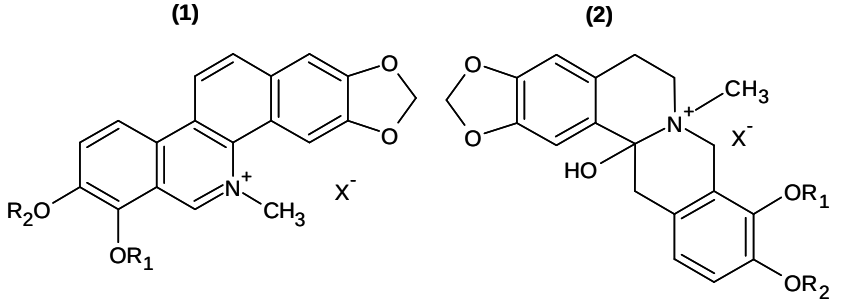



**Fig. 1.:** Chemical structures of cationic forms of **(1)** sanguinarine ($R_1$+$R_2$=$CH_2$) and chelerythrine ($R_1$=$R_2$=$CH_3$), **(2)** protopine ($R_1$+$R_2$=$CH_2$) and allocryptopine ($R_1$=$R_2$=$CH_3$).

## References

Kubala M., Vacek J., Popa I., Janovská M., Kosina P., Ulrichová J., Trávníček Z., Šimánek V., *J. Lumines.* 2011, *131*, 1340-1345.

Janovská M., Kubala M., Šimánek V., Ulrichová J., *Toxicol. Lett.* 2010, *196*, 56-59.

Vacek J., Vrublová E., Kubala M., Janovská M., Fojta M., Šimková E., Stýskala J., Skopalová J., Hrbáč J., Ulrichová J., *Electroanalysis* 2011, *23*, 1671-1680.

## Acknowledgements

This work was supported by the Ministry of Education, Youth and Sports (ME 10071) and by the Czech Science Foundation (303/09/H048 and P503/11/P312).

# 13
# CITP analysis of biominerals

Jarolímová Z., Pernica M., Lubal P.*
Department of Chemistry, Faculty of Science, Masaryk University,
Kotlářská 2, CZ-611 37 Brno, Czech Republic

CITP is an analytical electromigration technique suitable for cationic and anionic analysis. This contribution is concerning the simultaneous determination of anions (*e.g.* oxalates, phosphates) and metal ions (*e.g.* Ca(II), Mg(II)) which can be found in biominerals (*e.g.* renal stones).

The method optimization for separation and determination of analytes was carried out on electrophoretic equipment EA 102 (Villa Labeco, Spišská Nová Ves, Slovakia). The analytical procedure was optimized for simultaneous determination of mixture of anionic oxalate and phosphate species with detection limits: phosphate 0.9 μM, diphosphate 5.0 μM, triphosphate 1.9 μM, oxalate 0.7 μM while the detection limits for Ca(II) and Mg(II) ion determination were also estimated.

Then this procedure was applied for analysis of samples of renal stones (see Fig.). The developed approach can distinguish the kind of stone consisting of calcium phosphate and calcium oxalate[1]. This approach was also employed for the study of solubility of these compounds (calcium oxalate, hydroxyapatite) which are present in biominerals[1]

**Figure** Examples of renal stones

This method was utilized for the study of composition and solubility of those compounds in order to follow the experimental conditions having influence on their solubility. This procedure can be used for biomineral analysis as suitable alternative to other analytical methods (CZE, ionic chromatography). Also it can be used for study of physico-chemical processes leading to formation of these biominerals.

**References**

1. Königsberger, E., Königsberger, LC. *Biomineralization: Medical Aspects of Solubility*, Wiley, New York 2006.

**Acknowledgements**

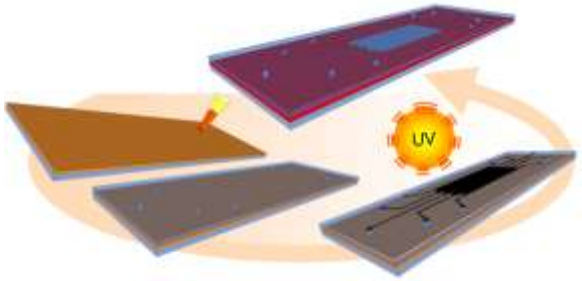
The work was supported by Ministry of Education of the Czech Republic (projects ME09065 and LC06035) and Grant agency of the Czech Republic (203/09/1394).

# 14
# Liquid phase lithography for rapid prototyping of microfluidic chips for free-flow electrophoresis, chemical sensing and chromatography

Jezierski S., Gitlin L., Nagl S., Belder D.*
University of Leipzig, Institute of Analytical Chemistry, Linnéstr. 3, 04103 Leipzig

The fabrication of microfluidic structures is demanding with regard to technical effort and production costs. Usually, meticulous and time consuming processes like structuring and bonding require a cleanroom facility.

With our new approach it is possible to produce microfluidic systems in a fast and economical way. A cleanroom is no longer necessary, and the technique can be repeated in every common laboratory, with only a printer and a UV light source being needed. The presented rapid prototyping procedure allows for the creation of different chip-layouts within a short time period. Furthermore bonding is not crucial anymore. After one photolithographic production step the created chips are ready to use and with a multi cycle process easy to modify (e.g. functional structures, surfaces).




To demonstrate the possibilities of this approach we present the production of a microfluidic free-flow electrophoresis (µFFE) chip. The challenge in producing structures for µFFE is to create a separation bed which is spatially detached from the electric contacts. Due to the chosen setup, resulting gases from electrolysis will not enter the separation bed. Nevertheless, the electric contact to couple the electric field is granted, which enables the electrophoresis. The presented chip was created using different polyethylene-glycol (PEG) diacrylates and one PEG methylether methacrylate in a photolithographic process. Thereby, it was possible to create a conductive wall between the electrodes and the separation bed. With the achieved chip it was possible to separate a mixture of fluorescent dyes and fluorescently labelled amino acids. Furthermore it was possible to integrate pH-sensor based on several of fluorescent dyes inside the µFFE structure. Additionally, we were able to expand the technique to produce robust chips for integration of chromatographic particulate and monolithic columns. These can be used for microfluidic chromatographic and electro chromatographic purposes.

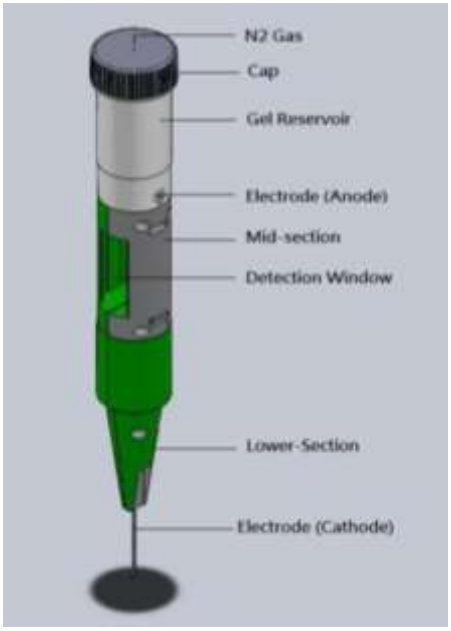
**References**

Jezierski, S., Gitlin, L., Nagl, S., Belder, D., *Analytical and Bioanalytical Chemistry*, DOI 10.1007/s00216-011-5351-2

# 15
# Rapid analysis of genomic DNA samples by a novel capillary gel electrophoresis platform

Kerékgyártó M.*, Bacskai I., Guttman A.
Horváth Laboratory of Bioseparation Sciences, Research Centre for Molecular Medicine, Medical and Health Science Center, University of Debrecen
H-4032 Debrecen, Nagyerdei krt 98, Hungary

Currently, most laboratories utilize slab gel electrophoresis systems as means of analysis of biopolymers such as nucleic acids and proteins. However, both PAGE and agarose slab gel electrophoresis methods are time consuming and labor intensive also need to be improved in terms of resolving power, throughput and cost per analysis. Capillary gel electrophoresis (CGE) has recently been emerged as an automated approach to solve this problem, offering rapid separations with high sensitivity, high-resolution, ruggedness and ease of operation.




Here we report on rapid analysis of genomic DNA samples by a novel single-channel capillary gel electrophoresis system utilizing LED based fluorescence detection and a pen size gel-electrophoresis cartridge that can automatically analyze DNA samples form 96 well plate. In this particular study, we have investigated 1000 genomic DNA samples for their purity and degradation stage in order to decide to use them in downstream QPCR analysis.

# 16
# Biomarker screening of the human plasma proteome with disease specific monoclonalantibodies

Kovács A.[1]*, Patai Z.[1], Sperling E.[1], Lázár J.[2], Balogh A.[2], Kádas J.[2], Szekrényes Á.[1], Takács L.[2], Kurucz I.[2] and Guttman A.[1,2]
[1]Horváth Laboratory of Bioseparation Sciences, Research Centre for Molecular Medicine,Medical and Health Science Center, University of Debrecen H-4032 Debrecen, Nagyerdei krt 98, Hungary
[2]BioSystems International Kft., H-4032 Debrecen, Nagyerdeikrt 98, Hungary

Monoclonal antibody proteomics is a novel platform based approach to discover proteins of biomarker potential.Starting from 500 mL normal pooled human plasma we have generated a comprehensive analyte library representing the human plasma proteome using specific chromatography based partitioning methods such as Blue Sepharose and thiophilic interaction chromatography to deplete the albumin and immunoglobulin fractions. This was followed by ammonium sulfate precipitation, gel filtration, cation and anion exchange as well as hydrophobic interaction chromatography fractionations steps. This process resulted in 783 fractions with the average protein concentration of 1 mg/mL. All chromatography and precipitation steps were carefully designed with the purpose of maintaining the native forms of the intact proteins throughout the fractionation process.

As a first application of the analyte library, a dot-blot assay was used to investigate and compare the separation routes of various disease specific acute phase proteins by monoclonal antibodies raised against them. In the majority of the cases the distribution of the antigens in the fractions matched the expectations predictable from the different phenomena – hydrophilicity-hydrophobicity, molecular shape, size – of the proteins.The library was also screened with mAbs raised against unknown protein antigens with the aim of the identification of potential biomarkers following the workflow of high throughput screening with Western blot verification and antigen identification by mass spectrometry.

**References**

Kovács, A., Sperling, E., Lázár, J., Balogh, A., Kádas, J., Szekrényes, Á., Takács, L., Kurucz, I., Guttman, A., Electrophoresis 2011, 32, 1916-1925.

# 17
# Iron oxide nanoparticle coating of organic polymer based monolithic columns for phosphopeptide enrichmentand off-line MALDI/MS or LC-ESI/MS/MS analysis

Křenková J.[1], Nováková K.[2], Zdráhal Z.[2], Foret F.[1]
[1]Institute of Analytical Chemistry of the ASCR, v. v. i., Brno, Czech Republic
[2]Core Facility - Proteomics, CEITEC-MU and Faculty of Science, Masaryk University, Brno, Czech Republic

Phosphoproteins are mostly characterized using mass spectrometry (MS) after proteolysis. However, phosphopeptides may be difficult to analyze by MS due to lower ionization efficiency of phosphopeptides in the presence of non-phosphorylated peptides. Therefore, efficient capturing or enrichment of phosphopeptides using selective affinity techniques prior to MS detection is necessary to increase ionization efficiency and detection sensitivity of phosphopeptides from highly complex peptide mixtures.

We have developed a new monolithic capillary column with an iron oxide nanoparticle coating for selective and efficient enrichment of phosphopeptides. Iron oxide nanoparticles were prepared by a co-precipitation method and stabilized by citrate ions. A stable coating of nanoparticles was obtained via multivalent electrostatic interactions of citrate ions on the surface of iron oxide nanoparticles with a quaternary amine functionalized poly(glycidyl methacrylate-co-ethylene dimethacrylate) monolith. A high dynamic binding capacity of 86 μmol per mL column volume was measured with adenosine-5'-triphosphate. Performance of the monolithic column was demonstrated with the efficient and selective enrichment of phosphopeptides from casein digests and off-lineMALDI/MS or LC-ESI/MS/MS analysis.

**Acknowledgements**
The Project is funded from the SoMoProprogramme. Research leading to these results has received a financial contribution from the European Community within the Seventh Framework Programme (FP/2007-2013) under Grant Agreement No. 229603. The research is also co-financed by the South Moravian Region. This work was also supported by the project “CEITEC - Central European Institute of Technology” (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund. Financial support from the Grant Agency of the Czech Republic (P301/11/2055,GD204/08/H054) and the institutional research plan (AV0Z40310501) is also acknowledged.

# 18
# Synthesis of a Caspase-3 sensor

Lišková M.* [1,2], Pazdera P.[2], Klepárník K.[1], Foret F.[1]
[1]Institute of Analytical Chemistry ASCR, v. v. i., Brno, Czech Republic
[2]Masaryk University, Faculty of Science, Centre for Syntheses at Sustainable Conditions and their Management, Brno, Czech Republic

Caspase 3, a cysteine-aspartic acid protease activated in the apoptotic pathway, plays an essential role in the programmed cell death. Moreover, failure of apoptosis is one of the main contributions to tumor development. Under normal circumstances, caspases recognize tetra-peptide sequences Asp-Glu-Val-Asp (DEVD) on their substrates and hydrolyze peptide bonds after aspartic acid residues. Activity of Caspase-3 is usually assayed by a chemiluminescence reaction. The best limit of detection by using commercial methods is higher than 1pg of Caspase 3.

We have constructed a special device for the determination of Caspase-3 activity in free cells. The activity of Caspase 3 is determined by the system based on Luciferin/Luciferase chemiluminescence reaction. The luciferin modified with tetrapeptide sequence (DEVD) specific to the recognition for Caspase-3 is cleaved to form free luciferin, which immediately reacts with luciferase to produce light. Our detection device consists of a microfluidic chamber held inside a housing of photomultiplier module for photon counting (Sens-Tech P25USB) with spectral response 275-620 nm. The sensitivity of the device proved to be an order of magnitude more sensitive than currently available commercial technologies.

We have designed the synthesis of Caspase-3 sensor. The sensor consists of a CdTe quantum dot (QD), aderivative of Rhodamine B, caspase recognizing the tetra-peptide(DEVD) and linkers, bonding the sensor together. Common organic reactions are used for preparation of this sensor (substitution, double bond addition, reduction etc.), but with new organic approach based on piperazine and polyethylene glycol (PEG) chemistry. Optimization of the synthesis is investigated on several model reactions, where model reactants with the same functional group as the original component of the sensor are used. Products of synthesis are checked by NMR, FTIR, MS and CZE-LIF. Our sensor operateson Förster resonance energy transfer (FRET).QD as a donor chromophore transfers energy to an acceptor chromophore (derivative of Rhodamine) through non-radiative dipole–dipole coupling. Successful energy transfer changes the maximum emission wavelength before and after cleavage of DEVD sequence in the sensor. This sensor can be used for a sensitive determination of Caspase-3 concentration or activity.

**Acknowledgments**
This work was supported by The Grant Agency of the Czech Republic (GA203/08/1680, P301/11/2055 and P206/11/2377), Ministry of Industry and Trade (2A-1TP1/090) and institute research plan AV0Z40310501.

# 19
# A prospective non-invasive assessment of a human embryo viability based on capillary electrophoresis

Mádr A.[1*], Foltová K.[1], Žáková J.[2], Lousová E.[2], Crha I.[2], Glatz Z.[1]
[1]Department of Biochemistry, Faculty of Science and CEITEC, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
[2]Department of Gynecology and Obstetrics, Medical Faculty of Masaryk University and University Hospital, Obilní trh 11, 602 00, Brno, Czech Republic

The human embryo viability is mainly assessed by means of a light-microscopy in assisted reproduction. However, this extensively used technique have been confronted about its deficiencies and the world leading embryologist call for a novel method for an embryo quality assessment to improve an accuracy of an embryo viability determination. In general, *in-vitro* fertilization (IVF) procedures suffer from a relatively low rate of pregnancy thus multiple embryos are implanted to gain the higher pregnancy outcome. This strategy inevitably increases chances of a multiple pregnancy with associated health risk issues thus neonatal survival chances could be compromised and the mother is exposed to risks as well. Currently, elective single-embryo transfer policy is being preferred by IVF clinics thus improved methods for an embryo quality assessment are highly demanding. Besides invasive techniques like preimplantation genetic screening including biopsy, non-invasive assessment based on metabolome analysis of spent embryo culture media is very prospective and several analytical techniques were already tested for such purpose. Namely, $^{1}$H-NMR, LC-MS, LC-MS/MS and IR spectroscopy; however utilization of capillary electrophoresis (CE) was left behind.

Our research group examines the suitability of CE for targeted and untargeted metabolomics of spent embryo culture media. At first, applicability of CE for amino acids determination using the contactless conductivity detection was tried. Led by published hints [1-4], acetic acid in the mixture with neutral hydrophilic polymer serves as background electrolyte. Bare fused-silica capillary with an inner diameter 50 μm and a total length 80.0 cm (65.0 cm to the detection cell), constant cassette temperature 25°C and applied voltage +30 kV provide a complete amino acid profile of commercially available G-1™ medium (Vitrolife, Göteborg, Sweden). Advantages and drawbacks are discussed and possible improvements are outlined.

**References**

1. Coufal, P., Zuska, J., van de Goor, T., Smith, V., Gas, B., *Electrophoresis* 2003, 24, 671-677.
2. Tuma, P., Samcova, E., Andelova, K., *J. Chromatogr. B* 2006, 839, 12-18.
3. Tuma, P., Soukupova, M., Samcova, E., Stulik, K., *Electrophoresis* 2009, 30, 3436-3441.
4. Tuma, P., Samcova, E., Stulik, K., *Anal. Chim. Acta* 2011, 685, 84-90.

**Acknowledgements**

Authors would like to express thanks for the financial support provided by the Czech Science Foundation (Project No. P206/11/0009) and by the Ministry of Education, Youth and Sports of the Czech Republic (Project MSM 0021622413 and Research Centre LC06023).

# 20
# Important electromigration effects of carbon dioxide in capillary electrophoresis at high pH

Malá Z., Gebauer P., Pantůčková P., Boček P.

Institute of Analytical Chemistry of the Academy of Sciences of the Czech Republic, v.v.i., Veveří 97, CZ-602 00 Brno (Czech Republic)

This paper reveals the importance of omnipresent effects of carbonate in capillary zone electrophoretic analyses of anions in alkaline background electrolytes and brings the knowledge necessary to reach correct qualitative and quantitative results. Computer simulations and experimental study of selected model systems with indirect UV absorption and conductivity detection have shown that carbon dioxide absorbed from air into BGEs and samples induce important electrophoretic effects like formation of new additional zones and/or boundaries that may further induce strong and pronounced temporary changes in the migration of analytes. Examples are reduction of the pH of alkaline BGEs around pH 11 by up to 1 unit or formation of a pronounced detectable carbon dioxide peak comparable with peaks of analytes at 1 mM level. The higher the pH of the BGE, the stronger these effects and the broader their spectrum, involving (i) changes of effective mobilities and selectivity due to changes in pH of the BGE, (ii) occurrence of additional system zones appearing in form of peaks, dips or more complex disturbances in the detection signal, (iii) temporary interactions with the sample components and subsequent modification of the separation process and of its result.

**Acknowledgements**
We gratefully acknowledge support by the Grant Agency of the Academy of Sciences of the Czech Republic (Grant No IAA 400310703), by the Grant Agency of the Czech Republic (Grants No 203/08/1536 and P206/10/1219) and by the Institutional Research Plan AV0Z40310501 of the Academy of Sciences of the Czech Republic.

# 21
# Determination of disinfection by products in drinking water on an electrophoresis chip

Masár M., Danč L.*, Žurek R.†
Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynska dolina CH-2, SK-84215 Bratislava, Slovakia

A development of capillary electrophoresis (CE) methods on the chip for separation and determination of some nonvolatile disinfection by-products formed during the chlorination of drinking waters was the aim of this work. Monochloroacetic, dichloroacetic, trichloroacetic, monobromoacetic and dibromoacetic acids were studied in this context. Halogenacetic acids (HAA) are toxic to humans, plants, and in particular to algae. Some of them are carcinogens.

Separations were realized on the column-coupling (CC) chip with conductivity detectors placed at the end of each separation channel. Zone electrophoresis (ZE) separations carried out in the 2$^{nd}$ separation channel were combined with electric field sample stacking or isotachophoresis (ITP-ZE) pre-concentration of the analytes. ZE separations of HAA were studied in different carrier electrolytes at pH interval 4-6. In ITP-ZE separations of HAA, ITP as a sample injection technique for ZE on the CC chip was used. ITP separations were carried out at low pH (pH 4) to remove potential interfering constituents (weak acids) present in drinking water.

RSD values of migration times for studied analytes were in the range of 0.1-2.7%, and for peaks areas they were between 1.2-9.9%. The limits of detection (LOD) for HAA were in the range of 45-180 μg/l when a 900 nl volume of the sample was loaded onto the chip. Recoveries of HAA in ZE and ITP-ZE determinations varied in 67-101% and 59-103%, respectively. Lower recoveries of HAA have been mainly caused by the analyte adsorption on the inner wall of the chip.

Different anions as potential co-migrating constituents present in drinking waters, e.g. chloride, fluoride, bromide, iodide, sulphate, oxalate, nitrate, nitrite, acetate and phosphate have been studied. Of these, only acetate and phosphate interfered in ITP-ZE determinations of HAA. A negative impact of these constituents in real water samples was eliminated by supported liquid membrane extraction (SLME) of HAA. In addition, this sample pretreatment technique allowed selective extraction and pre-concentration of the analytes.

**Acknowledgments**
This work was supported by grants VEGA 1/0672/09, UK/37/2011, VVCE 0070-07 and OPVaV-2009/4.1/02-SORO (CE Green II).

# 22
# Fast monitoring of cations and anions in water by chip electrophoresis

Masár M., Kruk P., Luc M.*, Kaniansky D.†
Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská Dolina CH-2, SK-84215 Bratislava, Slovakia
E-mail: luc@fns.uniba.sk

This work was focused on development of sequential CZE methods for determination of inorganic anions (chloride, nitrate, sulfate) and cations (ammonium, calcium, magnesium, potassium, sodium) in drinking waters. Two different background electrolytes (BGE) with corresponding separation mechanisms for each group of analytes (anionic and cationic BGE) were employed on the poly(methyl methacrylate) chip with conductivity detection.

Separations of anions were carried out at pH 4.2 in the BGE containing zwitterionic detergent. For cationic constituents, BGE with pH 3.2 containing wall adsorption suppressor (negatively charged surface of the chip) and 18-crown-6-ether was employed. In addition, water soluble high-molecular polymer (methylhydroxyethylcellulose) was used to suppress electroosmotic flow in both instances. Such conditions enabled separations of both groups of analytes. With respect to the sample injection volume applied (900 nl), the concentration limits of detection were estimated at 100 μg/L and 10 μg/L for anions and cations, respectively. Due to different concentration levels of the ions studied in real samples, maximum concentration ratios for the neighboring constituents were determined. Under preferred separation conditions (suppressed hydrodynamic and electroosmotic flow), the RSD values of migration times were below 0.8% for both groups of analytes. The peak areas of anions were characterized by 0.7-2.7% RSD while for the cations the RSD values of peak areas were in range 0.8-3.5 %.

A practical applicability of the developed analytical methods was demonstrated on the analyses of tap and packed drinking waters. Degassing and/or a proper dilution of the samples were the only pretreatment procedures before the analysis. The contents of anions and cations in drinking waters determined by the proposed CZE methods were in a good agreement with those claimed by water treatment companies. Total analysis time was approx. 15 min, corresponding to one parameter (ion) per 2 min, chip maintenance between the runs included.

**Acknowledgements**
This work was supported by the Slovak Grant Agency for Science (VEGA 1/0672/09), the Slovak Research and Development Agency (VVCE-0070-07), Comenius University (UK/307/2009) and the CEEPUS program CII-HU-0010-05-1011.

# 23
# CE-LIF of green fluorescent fusion proteins for investigation of phosphorylation

Michalcová L.[a], Dopitová R.[b], Hejátko J.[b], Válková M.[b], Preisler J.[a,b]
[a]Department of Chemistry, Faculty of Science and CEITEC, Masaryk University
[b]Research group Functional Genomics and Proteomics of Plants, Mendel Centre for Plant Genomics and Proteomics, CEITEC, Masaryk University

A histidine-containing phosphotransmitters from *A. thaliana* (AHP1-5) mediate signal transduction downstream from receptor histidine kinases to subsequent phospho-accepting response regulators via so called multistep phosphorelay. Auto-phosphorylated histidine of the histidine kinase becomes a substrate for the subsequent phosphorelay, leading via AHPs-mediated nucleus signal delivery to the activation of ARRs that are involved in transcription control or direct interaction with cytokinin response effector proteins.[1]

In this study, CE-LIF with 405 nm laser diode was used to study AHP5 fused with GFP. AHP5-GFP fusion proteins overexpressed in *E. coli* were used for initial optimization of CE-LIF technique because of lower concentrations of the fusion protein produced by plant. The stability of the AHP5-GFP sample was examined; the complex was found to be unstable with GFP being released. The GFP release was not affected by either a long-term storage at -80 °C and -20 °C or the addition of one of the inhibitors (EDTA, PMSF). These results were confirmed by the western blotting.

The phosphorylation of the sample AHP5-GFP *in vitro* is under investigation. Phosphorylation has not been successful yet, the reasons may be the sample contamination with proteins or an inappropriate phosphorylation protocol. Gel filtration was used to obtain mass fractions for better understanding electrophoregrams. Interestingly, some fluorescent fractions were not detected with the CE-LIF technique.

**References**

1. To, J.P.,Kieber, J.J. Trends Plant Sci. 2008, 13, 85-92.

**Acknowledgements**

This work was supported by The Czech Science Foundation (Grant No. 203/09/1025), by The Ministry of Education, Youth and Sports (MSM0021622415) and the project "CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund.

# 24
# Characterization of rhizobia by capillary electrophoresis, capillary isoelectric focusing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Moravcová D.*, Horká M., Šalplachta J., Kubesová A., Kahle V.
Institute of Analytical Chemistry of the AS CR, v. v. i., Veveří 97, 602 00 Brno, Czech Republic

Fast and reliable identification of individual bacterial strains is crucial for many branches of human activities as medicine, cosmetics, food industry as well as microbial forensics, environmental studies, or agriculture. Phenotypic identification of microorganisms using gram staining, culture, and biochemical methods is traditionally used; however, these methods are time-consuming, tedious, and low throughput to be applied to wide populations. In recent years, polymerase chain reaction has been used for sensitive and specific identification of bacteria species. Nevertheless, molecular techniques are expensive and technically demanding, therefore, there is still a request for other sensitive and valid methods for bacterial identification.

Capillary electrophoresis, capillary isoelectric focusing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry were used for analysis of bacterial cell suspensions of four strains of *Rhizobium*and one strain of *Sinorhizobium*species - *Rhizobium leguminosarum*bv. *trifolii*, *R. leguminosarum*bv. *viciae*, *R. galegae*, *R. loti*, and *Sinorhizobiummeliloti*. These bacteria belong to beneficial group of bacteria associated with crop plants and they can enhance plant growth directly by increasing nutrient availability.

All proposed techniques have shown differences in individual profiles of tested bacterial strains and especially capillary electrophoresis and capillary isoelectric focusing perform quick and effective bacteria characterization without any additional sample handling which reduces time necessary for the analysis.

**Acknowledgements**
This work was supported by the Grant Agency of the Academy of Sciences of the Czech Republic No. IAAX00310701, by the grant of the Ministry of the Interior of the Czech Republic No.VG20112015021, by the Ministry of Education, Youth and Sports of the Czech Republic by grant No. LC06023, and by the Institutional research plan AVO Z40310501.

# 25
# Electrophoretic separation of (2-aminoethyl)trimethyl-ammonium chloride hydrochloride derivatized oligosaccharides with contactless conductivity detection

Partyka J., Foret F.
Institute of Analytical Chemistry ASCR v. v. i., Brno, Czech Republic

Glycosylations are the most frequently occurring post-translational modifications of proteins. They play important roles in living organisms. Oligosaccharides released from proteins are typically analyzed after a separation step such as RP-HPLC or CE separation. For optical detection derivatization of saccharides by a chromophore or fluorophore, typically prior to the separation is necessary. Such labels are mostly designed with a charged group(s), which helps ionization for consecutive mass spectrometric analysis. Recently, Unterieser and Mischnick studied labeling of oligosaccharides with a number of charged species for quantitative mass spectrometry[1]. Since the charged labels with quarternary ammonium group were also interesting for capillary electrophoretic separations we have tested such an approach. The aim of our work was obtaining a powerful tool for electrophoretic separation with good electrospray ionization. The tested sample was dextran ladder prepared by partial hydrolysis of dextrans from *Leuconostoc mesenteroides* (average molecular weigh = 9000-11000 g/mol). This dextran ladder together with 6´-sialyl-D-lactose was derivatized by reductive amination with (2-aminoethyl)trimethylammonium chloride hydrochloride and separated by capillary zone electrophoresis and isotachophoresis. Separations were successfully performed with poly(vinylalcohol)-coated (PVA) capillaries free of electroosmotic flow with contactless conductivity detection – Fig.1.

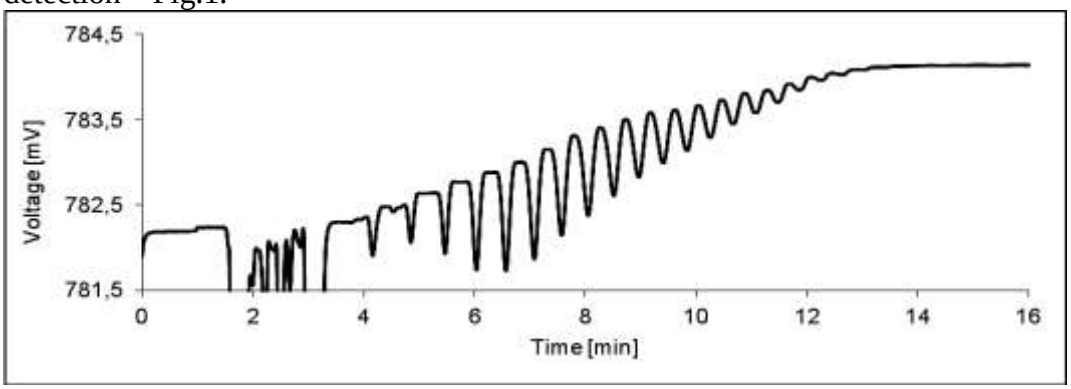



**Figure 1**. Capillary electrophoretic separation of AETMA-labeled dextran ladder with contactless conductivity detection.

## References

1. Unterieser, I., Mischnick, P., *Carbohydrate Research*, *346*, 68-75.

## Acknowledgements

This work was supported by the Grant Agency of the Czech Republic (P301/11/2055), the Ministry of Education, Youth and Sports (LC06023) and institute research plan AV0Z40310501.

# 26
# Separation of humic acids by zone electrophoresis and on-line combination of isotachophoresis- zone electrophoresis

Pastierová A.* [a], Bodor R. [a], Tesařová E. [b], Kaniansky D.† [a]
[a] Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina, 842 15 Bratislava, Slovakia
[b] Department of Physical and Macromolecular Chemistry, Faculty of Natural Sciences, Charles University in Prague, Albertov 6, 128 43 Praha 2, Czech Republic

Humic substances (HS) have an important role in the environment. These substances are classified into three groups, humic acids (HAs), fulvic acids (FAs) and humins, on the basis of their solubility in acidic and basic solutions. HAs and related fulvic acids (FAs) occur in soils, natural waters, marine and lake sediments, peat, lignin, brown coal and other natural deposits. Humic acids (HAs), natural compounds widely distributed in nature still of unknown structure, are intensively studied.
Different methods have been proposed for the separation, characterization and behavior of HAs, including capillary electromigration methods. Capillary zone electrophoresis (CZE) is the most used from capillary electromigration methods for the separation of HAs carried out in the open separation system. Effective combination of CZE with another electromigration method usually requires to use column coupling system. Column coupling system is provided by hydrodynamically closed system in many cases. The primary difference between open and closed hydrodynamic separation system is in the location of mechanical barriers at the end of the capillary (semi-permeable membrane or gel layer). The movement of the electrolyte solution in the capillary is mechanically limited by this way. Electroosmotic flow suppression in hydrodynamically closed system is required.
In our work we compared the separation of HAs carried out by on-line coupling of capillary isotachophoresis with zone electrophoresis (ITP-CZE) in a hydrodynamically closed separation system and by single-column zone electrophoresis with hydrodynamically opened separation systems. We used a zwitterionic detergent as a carrier electrolyte additive in CZE stage of ITP-CZE. The dodecyldimethylammoniopropanesulfonate (DDAPS) molecule, bearing the anionic and cationic groups (having no net charge), and in turn, the DDAPS micelles have no charges if they are present in the water. However, in an electrolyte, an imbalance between the anion- and cation- partition, can induces surface charges and surface potential. A critical micellar concentration of DDAPS is in the range 2-4 mmol/l.
A different electrophoretic pattern from the separation of HAs was reached by use of online combination of capillary isotachophoresis with zone electrophoresis and zone electrophoresis, respectively. A significant influence of DDAPS on the electrophoretic mobility of HAs was observed in both used method, and its addition to the carrier electrolyte leads to increased number of peaks in ITP-CZE.
Additionally, it was confirmed by spectrophotometry, that DDAPS increases the solubility of HAs in the water.

**Acknowledgements**
This work was supported by grants UK/39/2011 VEGA 1/0882/09, VVCE 0070/07, CII-HU-0010-05-1011

# 27
# New approach of sample preparation for sensitive determination of 8-hydroxy-2´-deoxyguanosine in human urine coupled with capillary electrophoresis

Petrů K.[1], Plíšek J.[1,2], Krčmová L.[1,2], Široká J.[1], Polášek M.[1]
[1]Department of Analytical Chemistry, Charles University in Prague, Faculty of Pharmacy in Hradec Králové
[2]Department of Metabolic Care and Gerontology, Teaching Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic
E-mail: klara.petru@faf.cuni.cz

8-hydroxy-2´-deoxyguanosine (8OHdG) belongs to the group DNA oxidative damage markers. It is believed to be excreted in urine without further metabolism and it´s urinary levels correlate with DNA damage. An extremely high concentrations were recorded at oncological patients and patients treated with radiation or chemotherapy, respectively. Thus determination of urinary 8OHdG has been proposed as a non-invasive assay of in vivo oxidative DNA damage [1].
A novel sensitive capillary electrophoretic method conducted with bubble cell capillary and applying large volume sample stacking was devised for on-line sample pre-concentration. A 106-fold enhancement of the analytical signal was achieved comparing with conventional 50 μm capillary.
Sample pre-treatment method Electromembraneextraction (EME) formedfrom Hollow fibre-liquid-phase microextractionconnected with power source isn't so frequent technique in clinical research. Application of the power source andtwoelectrodestothe conventionalmethodallows attracting chargedanalytestoward electrode of the oppositecharge throughsupportedliquidmembrane. In comparison with other extraction methods additional electrical field utilizing EME, yields to reducing time of the process.
EME will be applied to urine sample preparation andfurther analyte pre-concentration. Optimizing procedure such a choice of suitable fibre, organic solvent, working voltage and extraction time will be described. The results will be documented by an application of the whole analytical procedure to a patients urine samples.

**References**

[1] Mei, S. R., Yao, Q. H., Wu, C. Y., Xu, G. W., *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 2005, *827*, 83-87.

**Acknowledgements**

The study was supported by the Grant Agency of Charles University GAUK 263811/2011 and by the Project SVV 263 002.

# 28
# Potential of surface-enhanced Raman scattering detector for capillary electrophoresis

Přikryl J., Klepárník K., Foret F.
Institute of Analytical Chemistry of the ASCR, v. v. i.,
Veveří 97, 602 00 Brno, Czech Republic, prikryl@iach.cz

Surface-Enhanced Raman Spectroscopy (SERS) is interdisciplinary method covering vibrational spectroscopy, plasmonics and nanotechnologies. This method is based on the enhancement of the weak Raman scattering of molecules in a close proximity of metal nanomaterials, such as metal nanoparticles, roughened surfaces or metal tips. The enhancement factor could reach a value up to 1014 [1,2], and thus SERS becomes a high sensitive analytical method. In most of the applications, SERS was used as a stand-alone method. However, relatively little attention was focused to on-line coupling of SERS with separation methods such as liquid chromatography or capillary electrophoresis[3,4]. Nirodeet. al. published a simple way to integrate the on-column SERS detection in CE by usage of SERS-active silver nanoparticles suspended in the background electrolyte [3]. Leopold et. al. prepared a spot of SERS substrate by laser-induced citrate reduction of silver nitrate during the CE separation [4].

This poster is devoted to a new, simple photodeposition method of silver nanoparticles inside a fused-silica capillary induced by laser. The photodeposited compact spot of a size of ~10 μm is temporary and spatially stable and resistant to a hydrodynamic flow. The advantage of this approach is that neither the silver nanoparticles nor the chemicals for their preparation are components of the background electrolyte during the electrophoretic separation. Thus, the substrate formation and separation of analytes are two independent processes and can be performed under their optimum conditions. The zone broadening due to the sorption of analytes on the immobilized nanoparticles can be significantly reduced by an addition of 20% solution of methanol. The efficiency of capillary electrophoresis and detection selectivity of surface-enhanced Raman scattering is demonstrated by the 3D electropherograms of rhodamines 123 and B as model samples.

**References**

[1] Nie, S., Emory, S. R. *Science* 1997, *275*, 1102-1106.
[2] Kneipp, K., Wang, Y.,Kneipp, H., Perelman, L. T.,Itzkan, I.,Dasari, R. R., Feld, M. S. *Phys. Re .Lett.* 1997, *78*, 1667-1670.
[3] Nirode, W.F., Devault, G.L., Sepaniak, M.J., *Anal. Chem.* 2000, *72*, 1866-1871.
[4] Leopold, N., Lendl, B., *Anal. Bioanal.Chem.*2010, *396*, 2341-2348.

**Acknowledgements**

The research was financially supported by Grants of the Grant Agency of the Czech Republic GA203/08/1680, P301/11/2055, the Ministry of Education, Youth and Sports LC06023 and Research Plan of the Institute of Analytical Chemistry Z40310501.

# 29
# On-line inhibition study of cytochrome P450 2C9 isoform reaction with diclofenac

Řemínek R.*, Zeisbergerová M., Glatz Z.
Department of Biochemistry, Faculty of Science and CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

During last two decades, capillary electrophoresis (CE) proved its position of a powerful and versatile analytical technique for enzyme assays exhibiting a number of advantages over conventional methods. These include fast, highly effective separations, low sample and other chemicals consumption, and high throughput by automation. In addition, the fuse-silica capillary can serve not only as a separation column but also as a nanoscale reaction chamber integrating production, separation and detection of analytes into single run. In-capillary enzymatic assays thus further intensify the testing economy and enable fully automated analyses performed by commercially available CE devices.

Development of effective way for reactants mixing inside very limited space of a capillary constitutes a challenging task which prevents wider practical utilization of this attractive concept, nevertheless. Couple of approaches dealing with this limitation has been already presented; however, none of them is suitable for practical implementation. For this reason, the goal of this study was to introduce a generic methodology which enables interfusing of selectable reactants into homogenous reaction mixture inside a nanoscale capillary reactor. Principle based on combination of longitudinal and transverse diffusion was employed to fulfill these requirements. Conceptually, the solutions of reaction mixture components are injected by relatively low pressure as a series of consecutive narrow plugs having parabolic profiles due to laminar flow inside the capillary. Resulting character of plugs with rather longitudinal interfaces then enable creation of homogenous reaction mixture by both longitudinal and transverse diffusions within minute period.

On-line inhibition study of cytochrome P450 2C9 reaction with diclofenac and sulfaphenazole was performed in order to prove the applicability of proposed system. Obtained values of Michaelis constant, 50 % inhibitory concentration and inhibition constant were in agreement with literature data determined by other techniques. Presented principle thus constitutes a promising tool for on-line inhibition studies of cytochrome P450 2C9 requiring frequent changes in reaction mixture composition.

**Acknowledgements**
This work was supported by research project MSM0021622413 and research centre LC06023 both from Czech Ministry of Education and by grant No. P206/10/0057 from Czech Science Foundation.

# 30
# Electrophoretic and mass spectrometric analysis of the lipid A part of bacterial lipopolysaccharides

Sándor V.[1,*], Scheffer D.[1], Dörnyei Á.[2], Kilár A.[1], Kocsis B.[3], Kilár F.[1,2]
[1] Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary
[2] Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12, H-7624 Pécs, Hungary
[3] Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Pécs, Szigeti út 12, H-7624 Pécs, Hungary

Lipopolysaccharides (LPSs, or endotoxins) are the main components of the external membrane of Gram-negative bacteria. LPS is composed of three distinct structural regions: the O-chain polysaccharide, the core oligosaccharide and the lipid A moiety. Lipid A serves as the hydrophobic anchor of LPS in the outer membrane and is mainly responsible for the endotoxic activity. It generally consists of a β-1,6-linked glucosamine disaccharide backbone that is acylated by up to seven $C_{10}$-$C_{18}$ fatty acids or β-hydroxy-fatty acids linked as ester at C3 and C3' positions and as amides at C2 and C2' positions. The hydroxyl groups of these β-hydroxy-fatty acid chains can be further esterified by additional fatty acids. Phosphates, with or without other substituents, are linked at C1 and/or C4' positions. The number and type of the acyl chains and the state of the phosphorylation of the glycolipid are fundamental determinants of the toxicity of LPS.

The structure of lipid A is relatively conserved compared to the highly variable O-chain polysaccharide. The lipid A samples from a single bacterial population may contain more than one lipid A structural type. Alteration of lipid A structure (i.e., changes in acylation, phosphorylation, and glycosylation) could be affected also by the media for the growth of the bacteria. The moderate variability of lipid A molecules might serve as the basis for the rapid identification of bacterial strains.

The need for a fast and small-scale analysis of lipid A inspired us to develop a method that combines the high resolution efficiency of capillary electrophoresis and the high sensitivity and specificity of mass spectrometry. According to the chemical nature of the lipid A molecules we applied capillary electrophoresis coupled to electrospray ionization tandem mass spectrometry (CZE/ESI-$MS^n$), which might enable the separation and identification of these structurally very closely related amphiphilic compounds.

**References**

Hübner, G., Lindner, B. *Electrophoresis* 2009, *30*, 1808-1816.

**Acknowledgements**

Á.D. acknowledges the support of the János Bolyai Research Scholarship (Hungarian Academy of Sciences).

# 31
# SIMUL 5 COMPLEX - Simulation of the effects of complex-formation equilibria in electrophoresis

Svobodová J., Beneš M., Hruška V., Zusková I., Gaš B.
Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43, Prague 2, Czech Republic,
E-mail: svobod.j@seznam.cz

The simulation program Simul 5 [1, 2] that was developed in our laboratory enables to simulate processes in electrophoresis. The implemented theoretical model is based on principles of mass conservation, acid-base equilibria and electroneutrality. Simul 5 yields the complete picture of all the processes happening during separation and the concentration profiles of all electrolytes in time. Thus, the simulated electrophoregram (the detector record) can be obtained.

Currently, the theoretical model of electromigration was extended for systems containing complexation agent. The complexation equilibria were implemented into the previous model of electromigration. Simul 5 with complexation mode is especially useful for simulation of chiral separations, when a chiral selector is used as the complexation agent. It is applicable for any number of analytes at various degrees of dissociation. The model was verified experimentally. Three systems that differ in chiral selectors and/or experimental conditions were selected. The complexation constants and mobilities of complexes, which are the necessary input data for the new Simul 5 software, were determined. In the next step the simulations at different concentrations of chiral selectors were performed and compared with the experimental profiles obtained from UV and conductivity detections. The profiles show very good agreement in the position and shape of the analyte peaks. As the result, the newly developed Simul 5 with complexation mode can be used to predict the results of chiral separations and to study the processes taking place in electrophoresis systems, where complexation is involved in the separation.

**References**

[1] Hruska, V., Jaros, M., Gas, B., *Electrophoresis* 2006, *27*, 984–991.
[2] Hruska, V., Gas, B., Vigh, G., *Electrophoresis* 2009, *30*, 433–443.

**Acknowledgements**

Funding from the long term research plan of the Ministry of Education of the Czech Republic MSM 0021620857, the Grant Agency of the Czech Republic, grant No. 203/08/1428, the Grant Agency of the Charles University, grant No. 101309 and grant No. 323611 is gratefully acknowledged.

# 32
# Rapid identification of microorganisms using electromigration techniques and MALDI-TOF mass spectrometry

Šalplachta J.*[a], Horká M.[a], Kubesová A.[a], Moravcová D.[a], Růžička F.[b], Horký J.[c]
[a] Institute of Analytical Chemistry of the ASCR, v. v. i., Veveří 97, 602 00 Brno, Czech Republic, E-mail: salplachta@iach.cz
[b]Department of Microbiology, Faculty of Medicine, Masaryk University Brno, Czech Republic
[c]State Phytosanitary Administration, Division of Diagnostics, Šlechtitelů 23, 779 00 Olomouc, Czech Republic

Increasing demands request for rapid, reliable, and accurate analytical tool able to identify various types of microorganisms and their products are apparent in different fields of human activity. Especially, medicine and food industry have great interest in such techniques to ensure a patient or consumer safety because microbial outbreaks are spreading quickly. The conventional laboratory methods are still time-consuming and often inadequate for identification of phenotypically similar species. Therefore, the traditional phenotype systems are necessary to complement with multiple analytical techniques.

This work was focused on methods able to identify biofilm-negative and biofilm-positive strains of *Candida „psilosis“* as well as *Monilinia* species, respectively. For this purpose, isoelectric focusing (both capillary and gel format) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry were used. These techniques were applied to the analysis of either cell-surface proteins or whole cell suspension of representative strains of *Candida* and *Monilinia* species. Our results have revealed that proposed methods are suitable for rapid and reliable identification of *C. „psilosis“* strains in a common laboratory. Additionally, our results have shown that these fast techniques could be possible way for rapid and reliable differentiation of morphologically similar *Monilinia* species.

**Acknowledgements**
This work was supported by the Grant of Ministry of Interior of the Czech Republic no. VG20102015023 and by the Institutional research plan AVO Z40310501.

# 33
# Electromembrane extraction using stabilized constant d.c. electric current – a simple tool for improvement of extraction performance

Šlampová A., Kubáň P., Boček P.
Institute of Analytical Chemistry of the Academy of Sciences of the Czech Republic, v. v. i., Brno, Czech Republic

Electromembrane extractions (EME) are normally performed at constant voltage in all applications these days. This can be attributed to the fact that the only theoretical paper considered electric field as one of the key factors for the ion transfer in EME [1]. Another reason for the dominant use of constant voltage in EME may also be the fact that voltages used are mostly units to hundreds volts and usual constant d.c. voltage power supplies or even common 9V batteries [2] are used, which are readily available. Note, however, that extraction repeatability is often compromised for EME at constant voltage. Typical repeatability values (expressed as RSDs of peak areas) for a set of independent EME measurements are around 10%, however, significantly lower repeatability, with RSD values up to 30%, is often reported. This work presents an experimental approach for improvement of analytical performance of EME, which is based on the use of stabilized constant d.c. electric current. Extractions were performed using a high voltage power supply, which provided stabilized constant d.c. current down to 1 μA and facilitated current-controlled transfer of ions of interest from a donor solution through a supported liquid membrane (SLM) into an acceptor solution. Repeatability of the extraction process has significantly improved for EME at constant electric current compared to EME at constant voltage. The improved repeatability of the extraction process was demonstrated on EME-capillary electrophoresis (EME-CE) analyses of selected basic drugs and amino acids in standard solutions and in human urine and serum samples. RSD values of peak areas of the analytes for EME-CE analyses were about two-fold better for EME at constant electric current (2.8 – 8.9%) compared to EME at constant voltage (3.6 – 17.8%). Other analytical parameters of the EME-CE methods, such as limits of detection, linear ranges and correlation coefficients were not statistically different for the two EME modes. Moreover, EME at constant electric current did not suffer from SLM instabilities frequently observed for EME at constant voltage**.**

**References**

[1] Gjelstad, A., Rasmussen, K.E., Pedersen-Bjergaard, S. *J. Chromatogr. A* 2007, *1174*, 104-111.
[2] Kjelsen, I.J.O., Gjelstad, A., Rasmussen, K.E., Pedersen-Bjergaard, S. *J. Chromatogr. A* 2008, *1180*, 1-9.

**Acknowledgements**

Financial support from the Academy of Sciences of the Czech Republic (Institute Research Funding AV0Z40310501) and the Grant Agency of the Czech Republic (Grant No. P206/10/1219) is gratefully acknowledged.

# 34
# The direct electrophoretic determination of malondialdehyde in human plasma with sample stacking

Tůma P., Samcová E.
Institute of Biochemistry, Cell and Molecular Biology, Third Faculty of Medicine, Charles University, Prague, Czech Republic

Malondialdehyde is the major monitored marker of the oxidative damage of cell membranes in living organisms [1]. The determination of malondialdehyde is usually performed by spectrometry after the reaction with thiobarbituric acid. The thiobarbituric test suffers from the low specificity and overestimates plasma malondialdehyde level. Also several HPLC, GC and CE methods in combination with sample derivatization prior to separation step were developed [2,3].

In this study the direct electrophoretic method for the determination of malondialdehyde in human plasma without necessity of sample derivatization is introduced. Malondialdehyde is liberated from plasma proteins by alkaline hydrolysis in 0.5 M NaOH solution. Sample is subsequently neutralized by addition of sulfosalicylic acid, filtered and injected into the capillary. Large volume injection of sample is used to achieve low limit of detection at submicromolar levels. The electropherogram of human plasma with detailed experimental conditions are mentioned in Fig. 1.

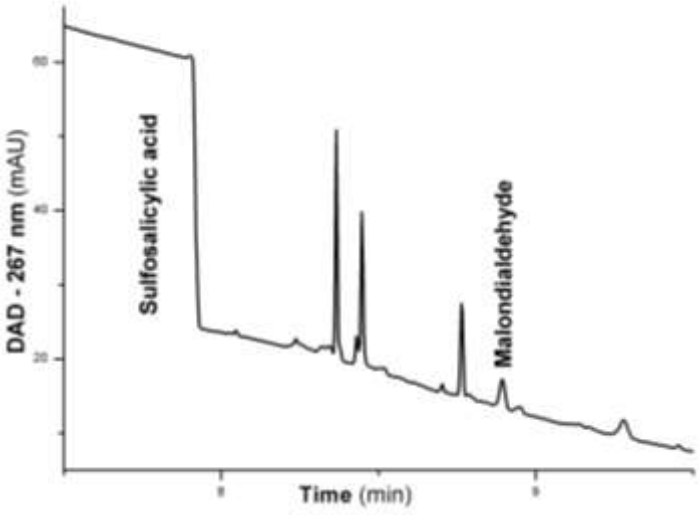



Fig. 1 An electropherogram of human plasma. Experimental conditions: background electrolyte 30 mM acetic acid/NaOH, pH 4.7; capillary total length 32.5 cm, length to detector 24 cm, id 50 μm; hydrodynamic injection 8000 mbar.s; separation voltage -15 kV; plasma preparation: 175 μL plasma + 25 μL 2 M NaOH, hydrolysis for 120 min at 60 ºC; addition of 200 μL water and neutralization with 35 μL of 3 M sulfosalicylic acid.

**References**

[1] Del Rio, D., Stewart, A. J., Pellegrini, N., *Nutr. Metab. Carbiovasc. Dis.* 2005, 15, 316-328.
[2] Mateos, R., Bravo, L., *J. Sep. Sci.* 2007, 30, 175-191.
[3] Matějčková, J., Samec, M., Jaček, M., Tůma, P., *Chem. Listy* 2011, 105, 375-380.

**Acknowledgements**

This work was supported by the Grant Agency of the Czech Republic, grants P206/10/1231 and P206/11/0707.

# 35
# Fast liquid chromatography as a tool for rapid screening of explosives

Šesták J.[1, 2], Moravcová D.[2], Večeřa Z.[2], Kahle V.[2]
[1] Institute of Analytical Chemistry of the ASCR, v. v. i., Veveří 97, 602 00 Brno, Czech Republic
[2] Faculty of Chemistry, Brno University of Technology, Purkyňova 464/118, 612 00 Brno, Czech Republic

Current devices for detection of explosives suffer by problems with selectivity and contamination. Coupling of liquid chromatography with ultra-sensitive chemiluminescence detection could be a right solution of these. Generally, explosive detection systems should respond instantly and for this reason a rapid LC method has to be developed and the use of very efficient separation columns [1] is necessary. In addition, the gradient elution can, of course, significantly increase performance of the system.

Submitted work assesses a suitability of columns filled with superficially porous particles [2], Kinetex 2,6 µm C-18, Poroshell 120, 2,7 µm C-18, and monolithic column Chromolith CapRod RP-18e to perform ultra-fast separation of target explosives (hexogen, octogen, trinitrotoluene and pentrit). Column permeability and efficiency in wide range of mobile phase velocities were evaluated. The results show that the columns filled with superficially porous particles are more efficient than the monolithic one but the difference is not dramatic and decrease of efficiency in higher mobile phase velocities is equivalent. On the other hand, the monolithic column reaches the best separation impedance [3].

Finally, columns filled with superficially porous particles as well as monolithic column can provide fast and high performance separation of explosives. The superficially porous particles are used when high efficiency and speed are a priority. However, if the pressure drop is limiting factor a monolithic column is a right choice. In this case, the tested monolithic column provided sufficient efficiency for resolution of target explosives in less than 2 minutes.

**References**

[1] Sýkora, D., Tesařová, E. *Chem. Listy*, 2007, 101, 190-199.
[2] Cunlife, J. M., Maloney, T. D. *J. Sep. Sci.* 2007, 30, 3104-3109.
[3] Bristow, P. A., Knox, J. H. *Chromatografia*, 1977, 10, 279-289.

**Acknowledgements**

This work was supported by the Institutional research plan AVO Z40310501. The financial support of the Ministry of Education, Youth and Sports of the Czech Republic by grant No. LC06023 is also gratefully acknowledged.

# 36
# A High-Throughput Platform for Preparation of APTS-Labeled N-Glycans: Improving the Accuracy, Reproducibility and Time-to-Results of N-Glycan Profiling

Szekrenyes A., Guttman A.
Horvath Laboratory of Bioseparation Sciences, Debrecen, Hungary

Kimzey M., Pourkaveh S., Mast S., Sun B., Minalla A., Haxo T., Nishida C., Wegstein J.
ProZyme, Inc., Hayward, CA, USA

Szabo Z., Karger B.L.
Barnett Institute, Boston, MA, USA

Chapman J.
Beckman Coulter, Inc., Brea, CA, USA

A novel sample preparation protocol is introduced for optional purification of mAbs including enzymatic deglycosylation, APTS labeling, sample cleanup, and standardization of N-glycans optimized for downstream CE-LIF analysis. The optimized labeling reagents provide complete derivatization with only one-hour incubation time, without degradation of important labile groups, such as sialic acid and core or outer-arm fucose residues. After labeling, the remaining unconjugated APTS is efficiently (>99.9 %) removed and the sample eluted in water, enabling the potential for mass spectrometry confirmation of atypical peaks. Spike-in, lower and upper internal mobility standards are used to normalize glycan migration times, further reducing variability between runs. Glucose unit (GU) assignment based on normalized glycan migration times showed significant improvement in precision compared to GU assignment without the use of internal mobility standards. It is envisioned that this unique glycoanalysis solution will be important for the standardization and expansion of N-glycan profiling in such large scale applications as clone selection and cell-culture optimization.

# 37
# Analysis of Altered IgG Galactosylation in Autoimmune Diseases

Váradi C., Rezsu N., Bertalan Meskó B., Guttman A.
Horváth Laboratory of Bioseparation Sciences, University of Debrecen, Hungary

Immunglobulins are the major secretory products of the immune system. The N-glycosylation on human immunoglobulins, especially on IgG1 (conserved site at Asn 297), plays critical roles in the bioactivity of these proteins. Significant differences have been reported in IgG glycosylation in pregnancy, aging and various diseases. The four characteristic glycans of IgG1 are biantennary-agalacto (G0), biantennary-monogalacto (G1 and G1') and biantennary–digalacto (G2) structures, with the relative amount of G0 showing the highest variability in the above mentioned conditions. The aim of this study was to investigate the changes in the relative amount of G0 glycans of IgG in a few prevalent autoimmun diseases and investigating the quantitative changes of G0 with transcriptomics data on galactosyl transferase expression. The glycosylation pattern in sex and age matched samples were analyzed by capillary electrophoresis to see the correlation between IgG N-glycan profile, galactosyl transferase expression and the pathogenesis of Rheumatoid arthritis and Crohn disease. IgG was isolated by means of Protein A affinity pulldown from the samples of relevant patients and the N-glycans were released by peptide-N-glycanase F (PNGase F). The released glycans were then fluorescently labeled with aminopyrene-trisulfonate (APTS) and analyzed by capillary electrophoreis with laser induced fluorescence detection. Significant differences were found in the galactosylation ratio after treatment between the responsive and non responsive patients.

# 38
# Capillary electrophoresis of CdTe quantum dots and its probes

Voráčová I.[1], Klepárník K.[1], Lišková M.[1, 2], Patrmanová M.[1,2], Zeiselová M.,[1] Foret F.,[1]
[1]Institute of Analytical Chemistry AS CR, v. v. i., Veveří 97, 602 00 Brno, Czech Republic, [2]Department of Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Quantum dots (QDs) are semiconductor nanocrystals with unique optical properties finding wide scope of applications. Water soluble CdTe QDs are prepared uncoated or coated with inorganic salts (CdS, ZnS, $SiO_2$) and with several organic ligands bonded on its surface. The organic ligands usually contain thiol groups that bind on QD surface and ionic groups (carboxyl or amino) that provide water solubility and serve as linkers for conjugation of other molecules [1, 2].
The QDs with mercaptopropionic acid organic ligand on the surface were conjugated via zero-length cross linkers 1-ethyl-3-(3-dimethyl-3-aminopropyl) carbodiimide hydrochloride and *N*-hydroxysulfosuccinimide with macrocyclic ligand (1,4,7-triacetyl-10-aminopenthyl- 1,4,7,10-tetraazacyclododecane, MPI) or its Zn(II) or Eu(III) ion complexes. Conjugation conditions were optimized and formation of the conjugate was confirmed by capillary electrophoresis with laser induced fluorescence detection (CE-LIF). The ratio of conjugated QD:MPI and QD:metal ion complex were also tested. The electrophoretic mobility increases from $-4.1 \times 10^{-8}$ to $-2.1 \times 10^{-8}$ $m^2V^{-1}s^{-1}$ for ratios QD: MPI 1:0 to 1:20. A further increase of this ratio has practically no influence on the mobility of conjugate. The QDs conjugated with simple organic molecules and its metal ion complexes should create luminescent probes for Förster resonance energy transfer (lanthanide (III) complex) and DNA labeling (Zn(II) complex). QDs with different sizes and organic ligands on the surface were characterized by CE-LIF using various background electrolytes. Their zeta-potential and charge were calculated from electrophoretic mobilities according to theory derived by Ohshima [3]. The determined charges in pH=10 are -12 in the case of 3.1 and 3.5 nm QD with mercaptopropionic acid and -14 in the case of 3.9 nm QD with thioglycolic acid on the surface. Providing the complete dissociation of organic ligands, the number of charges should also indicate the number of organic ligands bonded on QD surface.

**References**

[1] Kleparnik, K., Voracova, I., Liskova, M., Prikryl, J., *et al.*, *Electrophoresis* 2011, *32*, 1217-1223.
[2] Kleparnik, K., Liskova, M., Voracova, I., Hezinova, V., *et al.*, *Analytical and Bioanalytical Chemistry* 2011, *400*, 369-379.
[3] Ohshima, H., Journal of Colloid and Interface Science 2001, 239, 587-590.

**Acknowledgements**

This work was supported by grants of the Grant Agency of the Czech Republic (GA203/08/1680, P301/11/2055, P206/11/2377), Ministry of Education, Youth and Sports (LC06023) and institute research plan AV0Z40310501.

# 39
# The effect of different inactivation procedures on *Candida* species confirmed by capillary HPLC and CIEF

Vykydalová M.*[1,2], Horká M.[1], Moravcová D.[1], Růžička F.[3], Kahle V.[1]
[1] Institute of Analytical Chemistry ofthe AS CR, v. v. i., Veveří 97, 602 00 Brno, Czech Republic
[2]Department of Biochemistry, Faculty of Science, Masaryk University Brno, Kotlářská 267/2, 611 37 Brno, Czech Republic
[3]Department of Microbiology, Faculty of Medicine, Masaryk University, Pekařská 53, 602 00 Brno, Czech Republic

Three close species *Candida parapsilosis*, *Candida methapsilosis* and *Candida orthopsilosis* have different pathogenicity to humans. *C. parapsilosis* and *C. methapsilosis*existin two forms biofilm-positive and biofilm-negative. *C. orthopsilosis* does not form biofilm. C. "psilosis" occur on the skin, hands and mucous membranes of healthy people; however, biofilm positive strains especially *C. parapsilosis* become a significant problem for immuno-compromised patients leading even to death. Therefore, it is necessary to check the presence of infectious pathogens and to investigate possibilities of their inactivation. The subsequent control of an efficiency of inactivation procedure is important part of research as well.

In this study the efficiency of inactivation procedures on*Candida* species was investigated by capillary HPLC and CIEF. Biofilm-positivestrains of *C.parapsilosis* and *C.methapsilosis* were used as model organisms. Inactivation of these yeasts was achieved by different physical or chemical methods, e.g., boiling, using of 70% v/v ethanol or 4% v/v formaldehyde, or 2% v/v peroxoacetic acid [1]. Suspensions of cell pellets and supernatants after inactivation procedure were analyzed by CIEF and capillary HPLC.

Isoelectric fingerprints of tested bacteria cells have changed in all cases of inactivation compared to native cells. Significant differences in HPLC separation profiles of bacterial supernatants were found as well. Detailed study of the inactivation processes of different bacterial strains will be followed.

**References**

[1] Růžička, F., Horká, M., Holá, V., Kubesová, A., Pavlik, T., Votava, M., *Journal of Microbiological Methods* 2010, *80*, 299-301

**Acknowledgements**

This work was supported by the Grant Agency of the Academy of Sciences of the Czech Republic No. IAAX00310701, by the Institutional research plan AV0Z40310501, by the grant of Ministry of the Interior of the Czech Republic No. VG20112015021 and by the Ministry of Education, Youth and Sports of the Czech Republic by grant No. LC06023.

# 40
# In-capillary screening of matrix metalloproteinase inhibitors by capillary electrophoresis for coupling with ESI mass spectrometry

Wang* X., Adams E., Van Schepdael A.

Laboratory for Pharmaceutical Analysis, Faculty of Pharmaceutical Sciences, K.U. Leuven, Herestraat 49 PB 923, B-3000 Leuven, Belgium

Matrix metalloproteinases (MMPs) have been considered as a novel biomarker and potential therapeutic target in human cancer. The MMP-2 and MMP-9 which have been particularly implicated in tumor invasion and metastasis formation were selected as model enzymes. A capillary electrophoresis (CE) based method with enzymatic reaction inside the capillary for screening of MMP inhibitors with fluorescence detector was firstly developed in our lab. In order to develop a label free method for the screening of MMP inhibitors, we are trying to investigate the applicability of CE-MS for the in-capillary screening of MMP inhibitors. In this project, a volatile system was firstly developed with CE-fluorescence detector. The buffer concentration and pH have been optimized in this system. Finally, 20 mM ammonium acetate (pH 7.0) was selected for both enzyme reaction and electrophoretic separation. Then, the inline incubation was performed using this method. The whole reaction, separation and detection were achieved within one minute. For the mass spectrometry analysis, the ion trap MS conditions were optimized using reaction product. The negative ESI detection mode was selected. The sheath liquid composition was also optimized. Acetonitrile-water (80:20) was finally selected for further CE-MS investigation.

# 41
# Electrochemical investigation of flavonolignans and study of their interactions with DNA in the presence of Cu(II): Applications of *ex situ* voltammetric methods

Zatloukalová M.[a], Křen V.[b], Gažák R.[b], Kubala M.[c], Trouillas P.[d], Ulrichová J.[a], Vacek J.[a]

[a]) Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, CZ-775 15 Olomouc, Czech Republic, [b]) Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-142 20 Prague, Czech Republic, [c]) Department of Biophysics, Faculty of Science, Palacký University, 17. listopadu 12, CZ-771 46 Olomouc, Czech Republic, [d]) Université de Limoges, EA 4021, Laboratoire de Biophysique, Faculté de Pharmacie, 2 rue du Docteur Marcland, 87025 Limoges, France

Flavonolignans, silybin and its derivatives (2,3-dehydrosilybin, 7-*O*-methylsilybin, 20-*O*-methylsilybin) and isosilybin were studied using *ex situ* (adsorptive transfer, AdT) cyclic and square wave voltammetry (SWV). The two oxidation steps were described for flavonolignans at potentials $Ep_1$ +0.5 V and $Ep_2$ +0.85 V depending on experimental conditions (Fig. 1). The anodic currents of flavonolignans are related to their electron transfer processes (oxidation of hydroxyl groups), which was supported by density functional theory (DFT) and B3P86 theory level (Fig. 1).

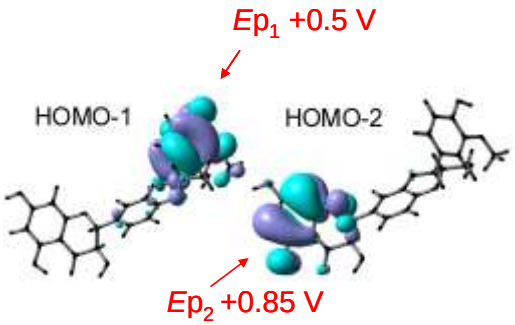



**Fig. 1**. Frontier orbital distribution and oxidation potentials in silybin.

The oxidation processes of flavonolignans can be affected by transition metal complexation. We found that silybin are able to chelate transition metals, especially $Cu^{2+}$. The electrochemical investigation of DNA interactions and damage caused in the presence of silybin/Cu complex and hydrogen peroxide is described.

**Reference**

Zatloukalová M., et al., *Bioelectrochemistry* 2011, *82*, 117–124.

**Acknowledgements**

This work was supported by the Czech Science Foundation (P301/11/0767 and 303/09/H048), by the Ministry of Education, Youth and Sports (MSM 6198959216) and by the Grant LF_2011_014.

# 42
# Optimization of non-aqueous media for capillary electrophoresis of dextromethorphan and its metabolites

Zeisbergerová M.*, Cvingráfová E., Glatz Z.
Masaryk University, Faculty of Science, Department of Biochemistry, Kamenice 5, 625 00 Brno, Czech Republic.

The use of organic solvents as the basis of the electrophoretic media in CE has been reported as a powerful technique for achieving highly selective and fast separations [1]. The selectivity of the non-aqueous capillary electrophoresis (NACE) is based on physicochemical properties of organic solvents, e.g. viscosity, electrical conductivity and acid-base behavior which are different from those of water. Non-aqueous background electrolytes (BGEs) also affect parameters of the CE system, namely electrophoretic mobility, acid-base and complexation characteristics. One of the most attractive features of NACE is the large panel of organic solvents to be used, in pure form or in mixture. Other advantages include reduced electric conductivity which results in low electric currents and Joule heat production. Notably, NACE extends the applicability of CE to analytes with poor solubility in water.

NACE was found to be a good alternative for the analysis of pharmaceutical drugs [2, 3]. In our work we have targeted the separation of dextromethorphan (DEX) and its metabolites. DEX is primarily used as an over-counter antitusive drug for temporary relief of cough. A variety of analytical methods have been developed to determine DEX and its metabolites in biological matrices, mainly involving the use of HPLC, GC, TLC and CE.

Our study has been focused on the influence of non-aqueous BGEs on the separation of DEX and its metabolites. Three organic solvents of different characteristics have been chosen for BGEs containing ammonium acetate; namely methanol, acetonitrile and 2-propanol. Apparent pH of all involved solutions was measured as well as the pH of aqueous electrolyte used for the comparison. Separations of the object analytes with the addition of levallorphan (internal standard) were performed in a 75 μm I.D. fused-silica capillary, 48.5/40 cm $L_{tot}/L_{eff}$, respectively. The evaluation was based on the resolution of all analytes, the efficiency of levallorphan separation, electroosmotic mobility and the total analysis time.

**References:**

1. Steiner, F., Hassel, M., *Electrophoresis* 2000, *21*, 3994-4016.
2. Geiser, L., Veuthey, J.-L., *Electrophoresis* 2007, *28*, 45-57.
3. Suntornsuk, L., *Anal. Bioanal. Chem.* 2010, *398*, 29-52.

**Acknowledgments**

Financial support from Grant Agency of Czech Republic (grant No. P206/10/0057) and Czech Ministry of Education (research project No. MSM0021622413 and research center LC06023) is gratefully acknowledged.

# 43
# Comparison of Two Electrophoretic Methods Documented by Separation of Dextromethorphan and its Metabolites

Zeisbergerová M., Langmajerová M*., Cvingráfová E., Glatz Z.
Masaryk University, Faculty of Science, Department of Biochemistry., Kamenice 5, 625 00 Brno, Czech Republic.

Capillary electrophoresis (CE) belongs to powerful analytical techniques and its great advantage is the existence of various modes. It allows analyses of different compounds from inorganic particles to biopolymers. CE is applied to drug assays because of its unique separation mechanism, speed, efficiency and versatility. Although capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography are the most commonly used modes for drug analysis, the usage of others (e.g. NACE, ITP, CEC) is increasing.

Non-aqueous capillary electrophoresis (NACE) is a term applied to describe a system in which pure organic solvents or mixtures thereof are used for background electrolytes. The application of organic solvents in CE has advantages over aqueous media such as good solubility of hydrophobic analytes, low conductivity and modification of separation selectivity. The organic solvent affects degrees of electrolyte dissociation, forms ion-pairs and changes conductivity of ions.

In our work we focused on the comparison of CZE and NACE methods applied to determination of a drug and its metabolites. As a model drug dextromethorphan was chosen. Parameters for CZE method were taken from the literature [1] and conditions for NACE were based on the publication [2]. Separations in both modes were carried out under these conditions: a fused silica capillary with inner diameter of 75 μm, 48,5/40 cm $L_{tot}/L_{eff}$, the positive separation voltage and UV detection at 200 nm. The methods were compared based on the following parameters: repeatability of migration times, normalized peak areas, peak resolution and total analysis time. Other parameters such as background electrolyte, sample matrix and working conditions will be discussed.

**Literature:**

1. Kristensen, H., *J. Pharm. Biomed. Anal.* 1998, *18*, 827-838.
2. Dong, Y., Chen, X., Chen, Y., Chen, X., Hu, Z., *J. Pharm. Biomed. Anal.* 2005, *39*, 285-289.

**Acknowledgements:**

The authors thank for financial support Grant Agency of Czech Republic (grant No. P206/10/0057) and Czech Ministry of Education (research project No. MSM0021622413 and research center LC06023).

# 44
# Irreversible protein heat denaturation analyzed by Bayesian approach

Žídková J.[a], Matejková M.[b], Žídek L.[b], Padrta P.[b], Bobálová J.[a], Sklenář V.[b]
[a] Institute of Analytical Chemistry of the ASCR, v. v. i., Veveří 97, 602 00, Brno, Czech Republic, zidkova@iach.cz
[b]National Centre for Biomolecular Research, Faculty of Science, and CEITEC, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic

Detailed description of heat denaturation is important for understanding protein function and for evaluating possibilities of industrial utilization of the protein. This presentation describes analysis of heat denaturation data, obtained by various experimental techniques, using Bayesian statistics. The first source of data was differential scanning calorimetry. NMR spectroscopy was used as a complementary approach to differential scanning calorimetry. One-dimensional NMR spectra reflect presence of well-defined three-dimensional structure of the studied protein and offer certain selectivity – analysis of the methyl and aromatic regions provides insight into the packing of the hydrophobic core of the studied enzyme, while the amide region reflects changes of backbone conformation. A denaturation curve can be obtained by recording a series of spectra at gradually increasing temperature. We present a protocol allowing to quantify the structural changes by a Bayesian analysis of the data and of a simple but correct model of irreversible denaturation. The procedure allows to evaluate enthalpic and entropic contributions in a case of sufficiently sampled temperatures. The methodology is presented on an example of non-specific lipid transfer protein 1 from barley. This protein denaturates irreversibly at extremely high temperatures (approximately 110°C) and thus presents a considerable experimental challenge. In order to monitor protein behaviour as such high temperatures, a special set-up (sealed tubes and choice of a suitable probe-head) was used.

**References**

1. Matejková, M., Žídková, J., Žídek, L., et al., *JAFC* 2009, *57*, 8444-8452

**Acknowledgments**

This work was supported by the Grants 1M0570 (J.Ž. and J.B.), MSM0021622413, (M.M. and L.Ž.) and LC06030 (V.S.) of the Ministry of Education, Youth, and Physical Culture of the Czech Republic and by Grant AV0Z40310501 of the Academy of Sciences of the Czech Republic (J.Ž. and J.B.).

# 45
# Analysis of cellular metabolites by HILIC UHPLC-MS/MS

Župková M., Janečková H., Fikarová I., Najdekr L., Wojtowicz P., Mičová K., Friedecký D., Adam T.
Laboratory of Inherited Metabolic Disorders and Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic

**Background:** Metabolomics became a valuable tool for diagnostics and research in clinical biochemistry over the past few years. Analysis of human metabolome is still a great challenge thanks to the variability of the compounds and due to the huge amount of metabolites. Development of methods is necessary for better understanding of all metabolic processes. With new more faster and precise methods we will be able to analyse and find more metabolite markers.

**Methods:** Separations were performed on an Ultimate 3000 RS UHPLC (Dionex) with aminopropyl column (Luna 3 μm NH2, 2 x 150 mm, Phenomenex). The mobile phase consisted of 20mM ammonium acetate (pH 9.45) as eluent A and acetonitrile as eluent B. For detection, a QTRAP 5500 tandem mass spectrometer (AB Sciex) with electrospray ionization was used. The samples were measured in a multiple reaction monitoring mode in positive and negative mode. Dwell time was 15 msec with complete cycle of 3 sec. The method was tested on human leukocytes. Cells were quenched by the chloroform/methanol/water mixture (4:4:2.8). Final extracts were freeze-dried and re-dissolved in mobile phase. For accurate quantification 6 isotopically labeled internal standards were added to the samples before protein precipitation.

**Results:** We developed method including 352 intermediates of metabolic pathways based on Kegg and HMDB databases. The method covers aminoacids, organic acids, bases, ribosides, nucleotides, sugars, and alcohols. By this method all compounds were detected with the limit of detection in the range $10^{-8}$ - $10^{-5}$ mol/L. The analyses were completed within 72 minutes including both ionisation modes.

**Conclusion:** The developed method can be successfully used for profiling of polar metabolites and offers complementary approach to widely used separation systems based on reversed phase columns.

**Acknowledgments**
This work was supported by grants IGA MZČR NT12218 and LF UP 2011-011.
The infrastructural part of this project (Institute of Molecular and Translational Medicine) was supported from the Operational programme Research and Development for Innovations (project CZ.1.05/2.1.00/01.0030).

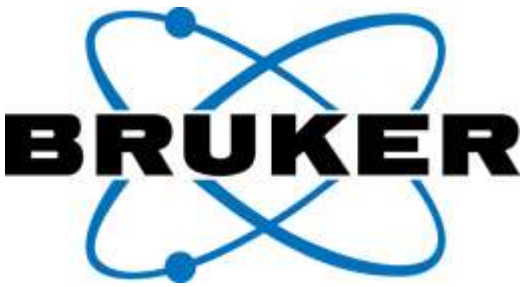
# SPONSORS





AKADEMIE VĚD
ČESKÉ REPUBLIKY



# NOTES

# ITP 2012, 19th International Symposium and Exhibit covering all aspects of Electro- and Liquid Phase-separations Techniques

## September 30 - October 3, 2012

Baltimore Inner Harbor
Baltimore, MD, USA

**ITP 2012 Symposium Chair**
Prof. Ziad El Rassi
Oklahoma State University
elrassi@okstate.edu

**Contact**
Ms. Janet Cunningham
ITP 2012
Symposium/Exhibit Manager
Barr Enterprises
janetbarr@aol.com
http://fp.okstate.edu/2012

**LAST year's ITP 2010 Symposium**
***Plenary & Keynote Lecturers***

Daniel Armstrong
Yoshinobu Baba
Doo Soo Chung
David Hage
Charles Henry
Jun Hirabayashi
Djuro Josic
Barry Karger
Vaclav Kasicka
Robert T. Kennedy
James Landers
Thomas Laurell
Bingcheng Lin
J. Michael Ramsey
Fred Regnier
Pier Giorgio Righetti
Steven Soper
Jonathan Sweedler
William Thilly
Jennifer Van Eyk
Timothy Veenstra
Edward Yeung

**The International Symposium on Electro- and Liquid Phase-separations Techniques is returning to the USA**

Renowned speakers from around the world will deliver cutting-edge presentations, discuss the latest developments in all aspects of electro- and liquid phase-separation techniques, provide in-depth discussion on electro- and liquid phase separations techniques, and on fundamental and practical issues in science and technology in multiple disciplines, and offer information to identify solutions to practical problems.

**The 3-day program of general and parallel sessions will be packed with 100+ talks, poster sessions, vendor workshops, courses, and an exhibition**

To build upon the momentum of last year's dynamic and most successful meeting in its series, the program will continue to offer a variety of themes encompassing electro- and liquid phase-separations (e.g., HPLC, Micro- and Nanoscale HPLC, two-dimensional electrophoresis, capillary and microchip electrophoresis, dielectrophoresis, micro- and nano-fluidics) in sessions such as

- proteomics
- glycomics
- various "omics"
- genetic analysis
- DNA sequencing and separation
- miniaturization and microfluidics
- bioanalysis
- biotechnology
- (bio)pharmaceutical analysis
- applications
- multidimensional separation approaches
- hyphenated techniques
- mass spectrometry as technology and its use in various omics
- novel instrumentation
- sample treatment
- and more!

The conference is a must to attend and will offer information that will help identify solutions to practical problems in areas including

- microfluidics
- DNA sequencing and separation
- multidimensional separation approaches and hyphenation to detection methods including mass spectrometry coupling (e.g., CE-, CEC- and LC-MS)
- proteomics
- glycomics
- genomics
- metabolomics
- foodomics

And other applications such as

- biotechnology
- (bio)pharmaceutical analysis and bioanalysis
- enantioseparations
- environmental
- food
- forensic
- clinical