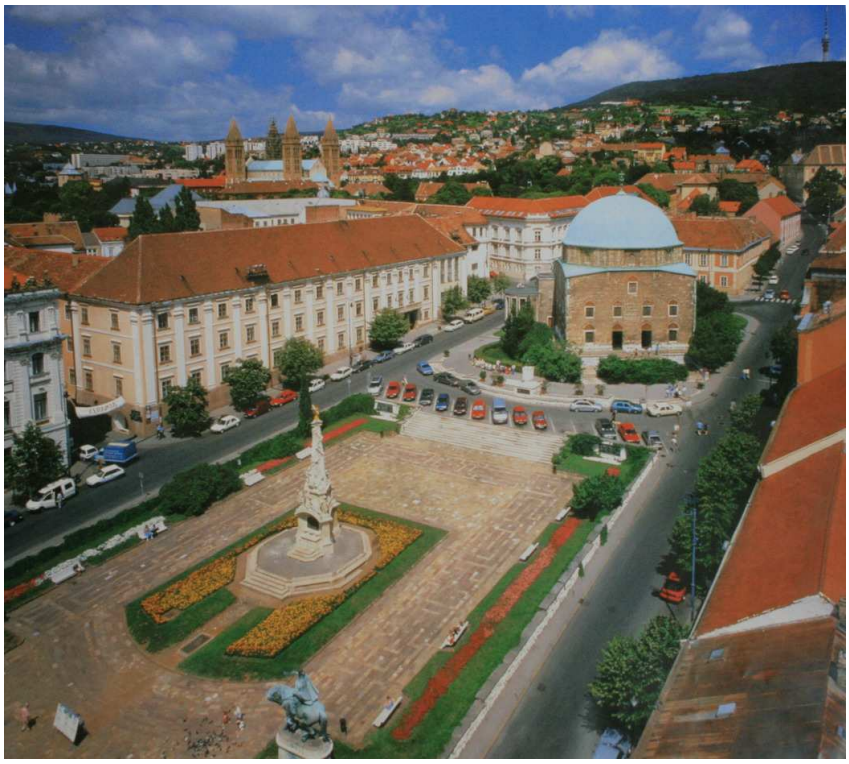
CECE 2009

6TH INTERNATIONAL INTERDISCIPLINARY MEETING ON BIOANALYSIS



PROGRAM AND ABSTRACTS

November 5 – November 8, 2009

PÉCS

HUNGARY

CECE 2009

6TH INTERNATIONAL INTERDISCIPLINARY MEETING ON BIOANALYSIS

Organized by
University of Pécs

PROGRAM AND ABSTRACTS

November 5 – November 8, 2009
PÉCS

HUNGARY

SPONSORS

NKTH
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PÉCSI TUDOMÁNYEGYETEM

EURÓPA KULTURÁLIS FŐVÁROSA 2010 – PÉCS

SIMKON KERESKEDELMI ÉS SZOLGÁLTATÓ KFT.

SIGMA-ALDRICH KFT.

WATERS KFT.,

UNICAM MAGYARORSZÁG KFT.

SPEKTRUM 3D KFT

Welcome to this year's CECE

This symposium is the sixth in the series of the traditional symposia organized previously in Brno, Czech Republic, starting with only a few lectures at the Institute of Analytical Chemistry in 2004. This year the symposium visits Pécs, the Cultural Capital of Europe in 2010.

Since its start it was the aim to create an interdisciplinary meeting for informal communication of scientists from different sides of bioanalytical sciences. The Symposium is now a serious member of the meetings of scientists, since it is continuously growing in research areas and number of participants, which gives great -

It is expected that this conference will further contribute to the exchange of ideas and will provide a forum for stimulating discussions.

The organizers want to thank you for your participation and hope that you will enjoy the scientific presentations, personal contacts and informal discussions.

http://cece2009.pte.hu

Organizing Committee

Ferenc Kilár - Chairman
Balázs Csóka
Ildikó Bíró
Borbála Boros
Anita Bufa
Ágnes Dörnyei
Attila Felinger
Ibolya Kiss
Ildikó Merk

Registration fee

The registration fee is 15000 HUF, which should be paid in advance (the bank transfer of the registration fee should be confirmed) or in cash at the Registration desk. An invoice will be provided at the Registration desk.

Social program

Welcome drink on Thursday evening, coffee breaks and lunches on Friday and Saturday, and the dinner on Friday are organized in the Restaurant of Hotel Hunyor (Jurisics Miklós utca 16.) for the registered participants.

The Ballet Performance ("Giselle") will take place in the National Theater (7621 Pécs, Színház tér 1.). Registration should be made at the Registration desk.

The Symposium dinner will take place in the Restaurant Cellarium (Hunyadi János utca 2. 7621 Pécs, http://www.diacell.hu/uzleteink_cellarium_restaurant.php)

Accommodation

The participants are accommodated at the Hotel Hunyor (7624 Pécs, Jurisics Miklós utca 16.) or Vasváry Villa (Regional Office of the Hungarian Academy of Sciences, 7624 Pécs, Jurisics Miklós utca 44.)

The accommodation between Thursday to Sunday (5-8 of November, 2009) is covered by the Symposium for all registered participants (whose registration has been acknowledged by the organizers). Other requests (longer stays) should be covered by the participant.

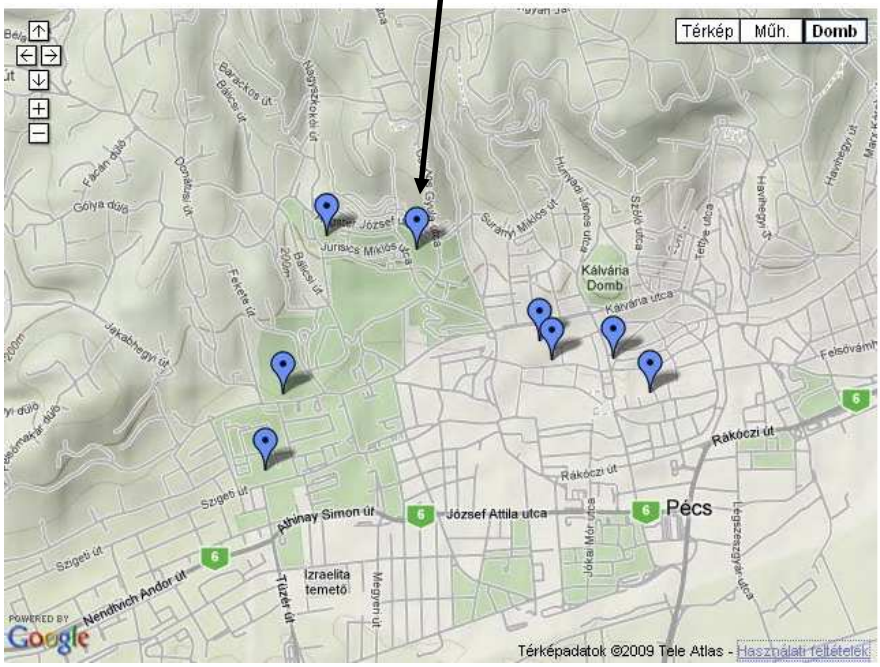
Venue

The symposium will be held at

HOTEL HUNYOR

H-7624 Pécs, Hungary

Jurisics Miklós utca 16**.**



http://cece2009.pte.hu

http://www.pte.hu/tartalom/401

Opening hours of the Registration desk

November 5, 2009: 15:00-19:00

November 6, 2009: 8:00-12:00

Language: English

Program

November 5, 2009

15:00 – 19:00 Registration
Hotel Hunyor, Pécs, Jurisics Miklós utca 16.

18:00 Welcome drink
Hotel Hunyor, Pécs, Jurisics Miklós utca 16.

November 6, 2009

8:00 – 12:00 Registration
Hotel Hunyor, Pécs, Jurisics Miklós utca 16.

09:00 Opening of the Symposium
Hotel Hunyor, Meeting Hall

09:15 **Cultural Capital of Europe 2010 - Pécs**

Chairman: Ferenc Kilár

09:30 **L-01** *Pier Giorgio Righetti*, Egisto Boschetti*
Politecnico of Milano, Milano, Italy
Plucking, pillaging and plundering proteomes with combinatorial peptide ligand libraries

10:00 **L-02** *Gabriel Peltre*, M. Poitevin, V. Dauriac, H. Li, Hélène Senechal, H. Chardin, P. Ponchet, S. Descroix*
ESPCI Paris France, Paris, France
Toward chip sized electrophoresis followed by immunodetection

10:30 Coffee break

Chairman: Tamás Janáky

11:00 **L-03** *András Guttman**
University of Debrecen, Debrecen, Hungary-USA
Deciphering the glycome by capillary electrophoresis

11:30 **L-04** *Jonas Bergquist*
Uppsala University, Uppsala, Sweden
On the hunt for novel biomarkers of degenerative diseases

12:00 **L-05** *Dubravka Matković-Čalogović, Biserka Prugovečki, Dalibor Milić and Ivica Đilović*
University of Zagreb, Zagreb, Croatia
X-ray crystallography as a tool for analysis of biological macromolecules

12:30 Lunch

13:30-15:00 **Poster discussion**
The authors are requested to be present at their posters during the Poster discussion

Chairman: Attila Gáspár

15:00 **L-06** *Christian Nilsson, Rickard Blom, Susane Rebiero, Ian Harwigsson, Staffan Birnbaum and Staffan Nilsson*
Lund University, Lund, Sweden
Nanoparticles in CEC

15:30 **L-07** *Tamás Janáky*, József Kovács, Judit Szeline Szomor, Dóra Simon, István Földi, Róbert Berkecz, Zoltán Szabó*
University of Szeged, Szeged, Hungary
Quantification of proteins in proteomics

16:00 **L-08** *Margit Cichna-Markl*
University of Vienna, Vienna, Austria
Multiplex real-time polymerase chain reaction for the simultaneous detection of food allergens

16:30 **L-09** *Gábor Dibó*
Eötvös Loránd University, Budapest, Hungary
Microwave-assisted organic synthesis: thermal or non-thermal effect

17:30 Dinner, Hotel Hunyor, Restaurant

19:00 “Giselle” Ballet Performance, National Theatre, (Színház tér 1.)

November 7, 2009

Chairman: Audrius Maruška

09:00 **L-10** *Dušan Kaniansky*, Marián Masár, Róbert Bodor, Jozef Marák*
Comenius University, Bratislava, Slovakia
Capillary and chip electrophoresis for column-coupling tools

09:30 **L-11** *František Foret*
Academy of Sciences CR, Brno, Czech Republic
Microseparations and mass spectrometry coupling, CECE Bioanalysis

10:00 **L-12** *M. Ryvolová, L. Krčmová, M. Akhter, J. Preisler, František Foret, P. Hauser, P. Maaskant and Miroslav Macka**
Dublin City University, Dublin, Ireland
Micro-separations need micro-light sources: LEDs and micro-LEDs as light sources of miniaturization

10:30 Coffee break

Chairman: Karel Klepárník

11:00 **L-13** *Audrius Maruška* and Olga Kornyšova*
Vyatautas Magnus University, Kaunas, Lithuania
Twenty years of continuous bed (monolithic) techniques: unsurpassed flexibility and success

11:30 **L-14** *A. Gáspár*, A. Nagy, I. Lázár, F. A. Gomez*
University of Debrecen, Debrecen, Hungary
Fabrication of microchips with chromatographic packings

12:00 **L-15** *Jozef Marák*, Andrea Stanova, Sona Gajdostinova and Dusan Kaniansky*
Comenius University, Bratislava, Slovakia
Potential of preparative isotachophoresis as a sample pretreatment technique for mass spectrometry

12:30 Lunch

13:30-14:00 **Poster discussion**
The authors are requested to be present at their posters during the Poster discussion

Chairman: František Foret

14:00 **L-16** *Karel Klepárník*, Jan Prikryl, Marcela Liskova, Ivona Svobodova, Vera Hezinova, František Foret*
Academy of Sciences CR, Brno, Czech Republic
Nanotechnologies in bioanalytical chemistry

14:20 **L-17** *Petra Jusková*, Veronika Ostatná, Emil Paleček and František Foret*
Academy of Sciences CR, Brno, Czech Republic
Fabrication and characterization of micro solid amalgam electrodes (μSAE) for bioanalytical applications

14:40 **L-18** *Tünde Angyal*, Lívia Nagy, Géza Nagy*
University of Pécs, Pécs, Hungary
Electrochemical methods for measurement of reactive oxidizing species

15:00 **L-19** *Ferenc Kilár*, Csilla Páger, Anna Takácsi-Nagy, Ágnes Dörnyei, Wolfgang Thormann*
University of Pécs, Pécs, Hungary
Capillary isoelectric focusing coupled to mass spectrometry

16:00 Guided Tour in Cella Septichora (Early Christian burial structure, which has been a World Heritage site since 2000) Pécs, Szent István tér

18:30 Symposium Dinner, Cellarium Restaurant (H-7621 Pécs, Hunyadi út 2.)

November 8, 2009

Departure

LIST OF POSTERS

P-01 *V. Atanasov*, I. Andreeva, S. Petrova*
Sofia University \"St. Kliment Ohridski\", Sofia, Bulgaria
Isolation of pharmacologically active proteins affecting blood coagulation from Bulgarian viper venom (*Vipera ammodytes meridionalis*)

P-02 *Katalin Balogh*, Peter Ács, Mónika Szili, Barna Kovács*
DDKKK Zrt., Pécs, Hungary
Anion-effect on the spectroscopic propreties of mercury containing fluorescein derivatives

P-03 *Róbert Bodor, Vladimíra Jánošová, Dušan Kaniansky*
Comenius University, Bratislava, Slovakia
Using discrete spacers as enhancing the resolution in capillary zone electrophoresis with coupled by capillary isotachophoresis

P-04 *Orsolya Bouquet*, Ildikó Kustos, Béla Kocsis, Ferenc Kilár, Tamás Lóránd*
University of Pécs, Pécs, Hungary
Examination of the protein profile of *Candida albicans*: effect of fused mannich ketons

P-05 *Marcin Cichosz*, Maciej Walczak, Roman Buczkowski, Justyna Sońdka*
Nicolaus Copernicus University, Toruń, Poland
Application of five-parameters Weibull equation for determination an average concentration of methane in biogas from corn silage in the periodic methane fermentation process

P-06 *Marcin Cichosz, Maciej Walczak**
Nicolaus Copernicus University, Toruń, Poland
Evaluation of the carbon to methane conversion coefficient during the anaerobic fermentation process of selected maize silages

P-07 *Ladislav Dančˇ*, Marián Masár, Dušan Kaniansky, Peter Turčáni*
Comenius University, Bratislava, Slovakia
Direct determination of organic acids in celebrospinal fluid by zone electrophoresis as used a column-coupling chip technology

P-08 *Ágnes Dörnyei*, Anikó Kilár, Annamária Bui, Zoltán Szabó, Béla Kocsis, Ferenc Kilár*
University of Pécs, Pécs, Hungary
Mass spectrometric analyses of lipopolysaccharides extracted from *Shigella sonnei* rough-type mutant strains

P-09 *Jarmila Dubajova, Zuzana Klöslova, Alžbeta Hegedűsova**
Constantine Philosopher University, Nitra, Slovakia
To assess the actual human exposure to PAHs generated from industrial, traffic and rural settings in Slovakia

P-10 *Nasim Ghasemzadeh*, Stellan Hjertén and Fred Nyberg*
Uppsala University, Uppsala, Sweden
Application of artificial gel antibodies for detection and quantification of biomarkers in clinical samples

P-11 *Róbert Góra*, Roharik Pavol, Milan Hutta*
Comenius University, Bratislava, Slovakia
Orthogonal off-line combination of RP-HPLC and SEC for analysis and characterization of selected enviro-biopolymers

P-12 *Radoslav Halko*, Tibor Neurocny, Milan Hutta*
Comenius University, Bratislava, Slovakia
Fractionation of environmental biopolymers by immobilized aluminium(III) metal ion affinity chromatography

P-13 **Hammoudi K.; Kord A ; Douali S*
University of Boumerdes, Boumerdes, Algeria
Analysis of some components extracted from *Scilla maritima* growing in the North of Algeria

P-14 *Blanka Hégrová*, Jan Preisler*
Masaryk University, Brno, Czech Republic
Chromatographic (RP-HPLC) analysis of cholesterol and selected phytosterols

P-15 *Ondrej Hegedűs, Vladimír Pavlík, Zuzana Šmotláková, Jarmila Dubajová, Alžbeta Hegedűsová*, Silvia Jakabová*
Regional Authority of Public Health, Nitra, Slovakia
Evaluation of the equivalence of HPLC method and the standard spectrophotometric method for determination of creatinine

P-16 *Michal Horčičiak, Marián Masár, Peter Bel, Dušan Kaniansky*
Comenius University, Bratislava, Slovakia
A direct trace determination of glyphosate in drinking water using column-coupling electrophoresis chip with conductivity detection

P-17 *Abdullah Ijaz Hussain, Farooq Anwar*, Abdul Jabbar, Shahid Mahboob and Poonam Singh Nigam*
University of Agriculture, Faisalabad, Pakistan
Seasonal variation in the composition and biological activities of essential oil of *Rosmarinus officinalis* from Pakistan

P-18 *Silvia Jakabová*, Ivan Baláž, Imrich Jakab*
University of Pécs, Pécs, Hungary
Occurrence of risk elements Cd and Pb in small terrestrial mammals from Upper Nitra region

P-19 *Omar Kaddour* and Khaled Hammoudi*
University of Boumerdes, Boumerdes, Algeria
Validation of HPLC method for quantitation ketoprofene in Ketoprofenid suppository

P-20 *Ibolya Kiss*, Ivett Bacskay, Ferenc Kilár and Attila Felinger*
University of Pécs, Pécs, Hungary
Mass transfer on superficially porous and totally porous reversed phases in HPLC

P-21 *Andrej Krafcik*, Melania Babincova and Peter Babinec*
Comenius University, Bratislava, Slovakia
Analysis of trajectory of magnetic particles in magnetic fields for cell separation and transfection

P-22 *Pavol Kruk*, Marián Masár, Dušan Kaniansky*
Comenius University, Bratislava, Slovakia
Zone electrophoresis separations of aliphatic and aromatic amines on a polymethylmethacrylate chip

P-23 *Sándor Kunsági-Máté,* Sophie Lecomte, Erika Ortmann, Éva Kunsági-Máté and Bernard Desbat*
University of Pécs, Pécs, Hungary
The effect of complex formation of thiacalix[4]arene with amino acids on the transition thermodynamics and kinetics of bovine serum albumin

P-24 *S. Lecomte, W. Van Grondelle, B. Desbat*, J.-L. Bruneel and C.Valéry*
University Bordeaux1, Pessac, France
Self association process of somatostatin characterized by Raman spectroscopy

P-25 *Marcela Lišková*, Karel Klepárník, Věra Hezinová, Ivona Svobodová, Jan Přikryl, František Foret and Pavel Pazdera*
Academy of Sciences CR, Brno, Czech Republic
Preparation and modification of quantum dots as selective probes for bioanalysis

P-26 *Milan Luc*, Marián Masár, Dušan Kaniansky*
Comenius University, Bratislava, Slovakia
Electrophoretic sample clean-up for zone-zone electrophoresis separations on a column-coupling chip

P-27 *Cornelia Majdik*, Andrada Măicăneanu, Cerasella Indolean, Silvia Burca, Maria Stanca*
Babes-Bolyai University, Cluj-Napoca, Romania
Cadmium removal from wastewaters using Ca-alginate immobilized bentonite as adsorbent

P-28 *Lilla Makszin*, Anikó Kilár, Béla Kocsis, Ferenc Kilár*
University of Pécs, Pécs, Hungary
Microchip electrophoresis for fingerprinting endotoxin chemotypes from whole-cell lysates

P-29 *Monica Marian*, Camelia Varga, Anca Peter, Leonard Mihaly Cozmuta, Silviu Birauas, Anca Mihaly Cozmuta*
North University, Baia Mare, Romania
Pollution of mushrooms and plant species used in soil mycoremediation

P-30 *Marián Masár*, Lenka Bachárová and Dušan Kaniansky*
Comenius University, Bratislava, Slovakia
(Ultra)trace determination of bromide in drinking water by capillary isotachophoresis-zone electrophoresis

P-31 *Anca Mihaly-Cozmuta*, Laura Bretan, Delia Boltea, Leonard Mihaly Cozmuta, Monica Marian, Anca Peter, Camelia Varga*
North University, Baia Mare, Romania
Traceability of heavy metals along of soil-plant-honey chain

P-32 *Leonard Mihaly-Cozmuta*, Anca Peter, Anca Mihaly-Cozmuta, Camelia Varga, Monica Marian, Delia Boltea*
North University, Baia Mare, Romania
Adsorption of different metal ions by a natural zeolitic tuff

P-33 *Livia Nagy*, Tünde Angyal, Adrienn Beidek, Szabina Geges, Martina Dóczy, Géza Nagy*
University of Pécs, Pécs, Hungary
Gathering physicochemical data for designing biofuel propelled grain dryers

P-34 *Csilla Páger*, Ágnes Dörnyei, Ferenc Kilár*
University of Pécs, Pécs, Hungary
Effects of the sandwich injection parameters on the pH gradient in CIEF analysis with MS detection

P-35 *Andrea Pastierová*, Róbert Bodor, Dušan Kaniansky*
Comenius University, Bratislava, Slovakia
Separation of humic acids by coupling capillary isotachophoresis with zone electrophoresis under micellar condition

P-36 *Daniela Georgiana Patrascu, Dan Enasescu, Constantin Mihailciuc, Ioan Stamatin, Lívia Nagy, Géza Nagy, Anton Alexandru Ciucu**
University of Bucharest, Bucharest, Romania
Electrocatalytic oxidation of dopamine and serotonine at carbon nanotube paste electrode modified with ferophthalocyanine

P-37 *Pavol Rohárik*, Róbert Góra, Milan Hutta*
Comenius University, Bratislava, Slovakia
Analysis and characterization of selected humic acids using RP-HPLC method

P-38 *Eliška Šišperová*, Eliška Glovinová and Jan Pospíchal*
Mendel University of Agiculture and Forestry, Brno, Czech Republic
Focusing of metals in ligand field step gradient

P-39 *Andrea Staňová*, Jozef Marák, Vitezslav Maier, Vaclav Ranc, Joanna Znaleziona, Juraj Ševčík and Dušan Kaniansky*
Comenius University, Bratislava, Slovakia
On-line combination of capillary zone electrophoresis with mass spectrometry used to analysis of buserelin in human urine

P-40 *Vishnya Stoyanova* , P. Tsvetkova, V. Atanasov, I.Tsacheva, S. Petrova*
Sofia University \"St. Kliment Ohridski\", Sofia, Bulgaria
Selection of phage-displayed recombinant scFv antibodies specific for sPLA2 from *Vipera ammodytes meridionalis*

P-41 *Petr Tůma* and Eva Samcová*
Charles University, Prague, Czech Republic
Capillary electrophoresis with contactless conductivity detection as promising tool for detection of inborn errors of organic acids metabolism

P-42 *Jindra Valentova, Renata Horakova, Iveta Pechova, Ferdinand Devínsky*
Comenius University, Bratislava, Slovakia
Gas chromatography-mass spectrometry determination of testosterone and its metabolite in urine

P-43 *Ágnes Varga*, Lívia Nagy, Géza Nagy*
University of Pécs, Pécs, Hungary
Determination of diffusion coefficients of glucose in solutions and gels by electrochemical TOF method

P-44 *Camelia Varga*, Anca Peter, Monica Marian, Leonard Mihaly-Cozmuta, Anca Mihaly-Cozmuta*
North University, Baia Mare, Romania
Protease and cellulase activity in the Bozanta mare tailing pond from Maramures county

P-45 *Réka Varga*, Aleksandar Széchenyi and Barna Kovács*
University of Pécs, Pécs, Hungary
Optical pH sensor based on a dual life time referencing (DLR) method

P-46 *Andrea Vargova*, Silvia Jakabova, Alzbeta Hegedusova, Ondrej Hegedus*
University of Pécs, Pécs, Hungary
Effect of soil reaction on selenium content in wheat in Nitra region

ABSTRACTS

LECTURES L-1 - L-19

L-01

PLUCKING, PILLAGING AND PLUNDERING PROTEOMES WITH COMBINATORIAL PEPTIDE LIGAND LIBRARIES

P.G. Righetti[1*], Egisto Boschetti [2]

[1]Politecnico di Milano, Department of Chemistry Materials and Chemical Engineering "Giulio Natta", Via Mancinelli 7, Milano 20133, Italy

[2]CEA-Saclay-DSV, iBiTec-S, 91191 Gif-sur-Yvette, France

Abstract

In any proteome, a few proteins dominate the landscape and obliterate the signal of the rare ones; most scientists lament that, in proteome analysis, the same set of abundant proteins is seen again and again. A host of pre-fractionation techniques has been described, but all of them are besieged by problems, in that they are based on a "depletion principle", often via immuno-subtraction (.e.g., in sera, by using a set of 6 to 12 antibodies against the most abundant species). Parasitic co-depletion removes thousands of low-abundance proteins, nullifying any attempt at bringing to the limelight the “unseen proteome”. A revolutionary approach consists in the "ProteoMiner Technology", a method enabling the capture of all species present in a proteome, but at much reduced protein concentration differences. This consists on a combinatorial library of hexapeptide ligands coupled to spherical porous beads. Such a vastly heterogeneous population of baits means that an appropriate volume of beads could contain a partner able to interact with all proteins present in a complex proteome. When these beads are contacted with proteomes of widely differing protein composition and relative abundances, they are able to “normalize” the protein population, by sharply reducing the level of the most abundant components while simultaneously enhancing the concentration of the most dilute species. Examples are given of analysis of human urine, sera and cerebrospinal fluid samples. In a red blood cell (RBC) lysate, where haemoglobin alone constitutes 98% of the total proteins, more than 1500 unique gene products have been found to constitute the remaining 2% proteome. By using this list of proteins, we have been able to decode a rare RBC disease, CDA II (congenital dyserythropoietic anaemia) in which the defective gene has been identified as the one coding for the *SEC23B* protein, located in chromosome 20. Additionally, these beads can be used to remove host cell proteins from purified recombinant proteins or protein purified from natural sources that are intended for human consumption, a matter of serious concern in the Bio Pharm. industry. These proteins typically reach purities of the order of 98%: higher purities often being prohibitively expensive. Yet, if incubated with "ProteoMiner beads", these last impurities can be effectively removed at a small cost and with minute losses of main, valuable product.

References

[1] Castagna, A., Cecconi, D., Sennels, L., Rappsilber, J., Guerrier, L., Fortis, F., Boschetti, E., Lomas, L., Righetti, P.G., *J. Proteome Res.* **4** (2005) 1917-1930.

[2] Righetti, P.G., Boschetti, E., Lomas L., Citterio, A., *Proteomics* **6** (2006) 3980-3992.

[3] Boschetti, E., Lomas, L., Righetti, P.G., *J. Chromatogr. A* **1153** (2007) 277-290.

[4] Antonioli, P., Fortis, F., Guerrier, L., Rinalducci, S., Zolla, L., Righetti, P.G., Boschetti, E., *Proteomics* **7** (2007) 1624-1633.

[5] Roux-Dalvai, F., Gonzalez de Peredo, A., Simó, C., Guerrier, L., Bouyssié, D., Zanella, A., Citterio, A., Burlet-Schiltz, O., Boschetti, E., Righetti, P.G., Monsarrat, B. *Mol. Cell. Proteomics* **7** (2008) 2254-2269.

L-02

TOWARD CHIP SIZED ELECTROPHORESIS FOLLOWED BY IMMUNODETECTION

Gabriel Peltre*, M. Poitevin, V. Dauriac, H. Li, Hélène Senechal, H. Chardin, P. Ponchet, S. Descroix

ESPCI Paris France, Paris, France

L-03

DECIPHERING THE GLYCOME BY CAPILLARY ELECTROPHORESIS

András Guttman[1,2], Zoltán Szabó[2], Marcell Olajos[3], Ákos Szekrényes[1] and Stefan Mittermayr[1]

[1]Horváth Laboratory of Bioseparation Sciences, University of Debrecen, Hungary
[2]Barnett Institute, Northeastern University, Boston, MA 02115
[3]Department of Analytical Chemistry, Pannon University, Veszprém, Hungary

There is a rapid recent progress in the research and understanding of the biological role of the carbohydrate moieties on glycoproteins, as part of the glycomics endeavor. Electric field mediated microseparation techniques, like capillary electrophoresis, capillary electrochromatography and microchip electrophoresis, in conjunction with fluorophore labeling of the released oligosaccharides of interest by 1-aminopyrene-3,6-8-trisulfonate (APTS) proved to be excellent tools to obtain very high resolution separation of complex mixtures of glycans. An automated glycoaffinity partitioning based sample preparation strategy was introduced to increase the specificity and sensitivity of capillary electrophoresis based analysis of glycosylation changes, both in relative migration index based glycan profiling and exoglycosidase mediated carbohydrate sequencing. We utilized high pressure (>10 kpsi) to accommodate PNGase F mediated release of glycans just in a couple of minutes. Using the optimized sample preparation and release conditions, examples of high performance capillary electrophoresis based N-linked glycosylation pattern analysis will be demonstrated. Other issues, such as removal of the large access of labeling reagent from the derivatization reaction mixture, elimination of the sugar content in biomarker studies from human plasma samples and the application of volatile buffer systems in exoglycosidase digestion mediated carbohydrate analysis is also addressed.

L-04

ON THE HUNT FOR NOVEL BIOMARKERS OF DEGENERATIVE DISEASES

Jonas Bergquist
Uppsala University, Natural Sciences, Analytical Chemistry, Department of Physical and Analytical Chemistry
jonas.bergquist@kemi.uu.se

L-05

X-RAY CRYSTALLOGRAPHY AS A TOOL FOR ANALYSIS OF BIOLOGICAL MACROMOLECULES

Dubravka Matković-Čalogović*, Biserka Prugovečki, Dalibor Milić and Ivica Đilović

Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia, dubravka@chem.pmf.hr

Abstract

X-ray diffraction analysis gives a lot of information about the molecular and crystal structure if single crystals of a compound can be obtained. For characterization of a compound, either a small molecule or a macromolecule, information about structure and properties is needed. Crstallization of proteins is often difficult and can be a bottleneck for such analysis. Some methods will be presented.

Our newest results in protein crystallography will be briefly discussed:

(1) enzymatic activity of tyrosine phenol-lyase (TPL) could be deduced by detailed structural analysis of native and mutated TPL forms in complexes with substrate analogues. Open and closed conformations were found in the structures giving insight into the enzymatic mechanism. Problems in defining molecular structure and formula of substrates from the electron density will be discussed;

(2) 3-hydroxyanthranilate 3,4-dioxygenase, an enzyme that catalyzes the conversion of 3-hydroxyanthranilate to quinolinic acid, has been extracted and purified from bovine kidney, crystallized, and its structure determined at 2.5 Å resolution;

(3) coordination of iron in bovine insulin changed upon addition of bromide or iodide ions into crystallization solutions.

L-06

NANOPARTICLES IN CEC

Christian Nilsson[1,2], Rickard Blom[1], Susane Rebiero[1], Ian Harwigsson[3], Staffan Birnbaum[2] and Staffan Nilsson[1*]

e-mail: Staffan.Nilsson@teknlk.lth.se

[1]Pure and Applied Biochemistry, Center for Chemistry and Chemical Engineering, Lund University, Sweden

[2]Biopharmaceutical Support QC, Manufacturing Development, Novo Nordisk A/S, Denmark

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Abstract

An important trend in chromatography has been towards smaller and smaller stationary phase particles, for example the development of UPLC. Another strategy using downsized particles is to suspend nanoparticles in the electrolyte and use them as pseudostationary phase (PSP) in CEC. [1, 2] In PSP-CEC, the stationary phase is used only once allowing fast column regeneration and circumventing carry-over effects. Nanoparticles possess a favorable surface-to-volume ratio and highly efficient separations can be foreseen. Dextran-coated nanoparticles were used in PSP-CEC achieving highly efficient separations of small, neutral analytes, i.e. dialkyl phthalates (up to 700 000 plates /m for retained analyte). [3] Furthermore, molecularly imprinted polymer nanoparticles with monoclonal behavior were synthesized and used for PSP-CEC separations of racemic propranolol, with no apparent tailing of the retained enantiomer. [4]

Currently, positively and negatively charged lipid-based nanoparticles (average diameter <100 nm) are used for protein separation at neutral pH and without organic modifier. Negatively charged lipid-based nanoparticles were used in PSP-CEC for separation of protein isoforms. Similarly charged, single amino acid substituted green fluorescent protein (GFP) mutants were separated utilizing high tricine concentrations to promote hydrophobic interactions. [5] Separation was performed in a capillary with effective length of 6.7 cm and efforts to transfer the separation systems to chip format are ongoing [6,7]. Preliminary results in Chip-based separations and UV-compatible non-scattering nano-particles will be discussed as well.

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L-07

QUANTIFICATION OF PROTEINS IN SOLUTION AND IN GEL

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Abstract

Quantitative results highly depend on what kind of methods and what type of standards are used for comparison. It is very difficult to determine the protein content even for a single protein containing sample. We used UV spectrophotometric, copper- and/or dye-based spectrophotometric methods and amino acid analysis (AAA) for determination of the amount of protein present in six pure, commercial protein products. Because of the various protein quantification methods based on different physical and chemical properties of the proteins the results are very diverse. We can recommend "AAA" as a gold standard to determine the absolute protein content of a pure protein standard.

Most common workflow applied in proteomics is 2D-electrophoretic separation, relative quantification based on spot's density followed by enzymatic digestion and mass spectrometric identification of proteins. There are many different mass spectrometric methods (applying heavy isotope as a label, or label-free) both for relative and absolute quantification of proteins in solution. A frequently observed issue is related to 2D-electrophoretic spots containing multiple proteins, in which case it is difficult to assign gel-based quantitative information to a single protein. We present results of our approach utilizing label free LC-MS methods to obtain absolute and relative quantitative information on proteins recovered from 1D and 2D gel-electrophoresis gels.

Nowadays many "in gel" digestion protocols are applied in proteomics which differ in number of washing steps, type of reducing and alkylating reagents, type of enzyme, duration and temperature of a particular reaction, solvents used for peptide extraction. Proteins are usually quantified by the comparison of the density of stained bands/spots (relative quantification). Main goal of our work was to determine which experimental conditions would result in the most robust identification and quantification over some model proteins. We present how the different parameters influence the efficiency of protein digestion, identification and quantification applying both "in solution" and "in gel" protocols. Protein digests were analyzed by Waters nanoAcquity UPLC/QTOF Premier LC-MS system. Label-free relative and absolute quantification of identified proteins was performed using ProteinLynx GlobalServer 2.3.

L-08

MULTIPLEX REAL-TIME POLYMERASE CHAIN REACTION FOR THE SIMULTANEOUS DETECTION OF FOOD ALLERGENS

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Abstract

Food allergies are an increasing health problem, particularly in industrialized countries. The actual prevalence of allergic reactions to food is estimated to be about 3% in adults and between 6 and 8% in young children. Clinical manifestations of food allergies comprise symptoms such as the so-called oral allergy syndrome, gastrointestinal, skin or respiratory disorders. Some foods can, however, induce severe life-threatening reactions, the most dramatic being the anaphylactic shock.

Currently, the only option for allergic individuals is to avoid the certain allergenic food completely. In many countries the presence of allergenic food ingredients has to be declared on the food label. According to European Union legislation 14 allergenic foods must be indicated in the list of food ingredients [1].

Analytical measurements have to be carried out to verify if allergen containing products are labelled in compliance with the regulations and if there are no hidden allergens in non-declared food products. Analytical methods developed so far are either protein based (e.g. enzyme linked immunosorbent assays (ELISAs)) or DNA based. In DNA based methods, a certain DNA sequence is amplified by the polymerase chain reaction (PCR) and detected either by agarose gel electrophoresis or – in so-called real-time PCR – by using fluorescence labelled probes.

In contrast to immunoassays, PCR has the potential of simultaneously amplifying several analytes in one reaction tube. Compared to common real-time PCR, so-called multiplex PCR has the advantages of saving time, reducing reagent costs and lowering the probability of cross-contamination. The main challenge in developing a multiplex PCR system is, however, to overcome the formation of primer dimers. In addition, optimum PCR conditions have to be found which enable to achieve comparable yields for each of the amplicons, independent on the concentration of the other amplicons.

The present lecture discusses the development and validation of multiplex real-time PCR methods for the simultaneous detection of food allergens and presents duplex PCR methods recently developed in our research group [2].

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L-09

MICROWAVE-ASSISTED ORGANIC SYNTHESIS: THERMAL OR NON-THERMAL EFFECT

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Abstract

For a decade microwave synthesizers have been around for enhancing chemical reactions in organic synthesis. At the frequency available for such instrumentation (2.45 GHz), molecular rotation occurs as molecular dipoles or ions try to align with the alternating electric field of the microwave. The rate of a chemical reaction is dependent on two factors: the frequency of collisions between molecules with correct geometry and the fraction of molecules having the minimum energy required to overcome the activation energy barrier. By using microwave irradiation, can molecules be at an energy level above the activation energy required for the reaction. Might this fact explain the observed dramatic increases in reaction rates?

We started to apply microwave-assisted synthesis technologies for the chemical synthesis of biologically active peptides. In the literature, side reactions have been well documented in solid-phase peptide synthesis, there has been concern that microwave, while speeding up reaction rates, may also accelerate racemization, aspartimide formation, etc. To compare, model peptides were synthesized at standard conditions, elevated temperature and under microwave conditions. Possible formation of side products were studied. Adaptation of new solvent combinations are also discussed.

L-10

CAPILLARY AND CHIP ELECTROPHORESIS FOR COLUMN-COUPLING TOOLS

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Abstract

CE equipments, reflecting a concept as proposed by Jorgenson [1], currently dominate for hydrodynamically opened separation systems and respecting for the single-column concept. The opened systems are not currently employed for capillaries over the 75-100 μm I.D.s or so. Such dimensions of capillaries are setting limits for: (a) low detectabilities of the analytes, (b) larger numbers of the analytes and (c) complex sample matrices. Only the capillaries with higher I.D.s, provided with a restrictor at the end of the capillaries [2], can be reduced for the limits mentioned. The opened system, while operating under an EOF transport might be restrictive for using ITP (see, a standstill in the separation system [3,4]).

The closed systems (see, e.g., instrumentation details in the book by Everaerts et al. [5]) can be taken to summarize (a) for the theory of electromigration processes [6] and (b) classified for the electromigration techniques [7]. However, this hydrodynamic concept requires suppressing for the EOF transport [5,6]. In addition, the closed systems have no restrictions while compared with the opened system (see, the previous paragraph). Surprisingly, the use of the closed systems is employed very seldom.

In fact, both the opened and closed separation systems are restrictive for the separation as based on the single-column technology. Using more advanced CE column systems solve these restrictions. For example, it is very benefiting by using the column-coupling system while including the column-switching run (CC-CE). However, the opened system is very complex to join the transport processes (i.e., the hydrodynamic, EOF and electromigration transports), especially, as these might have very significant velocity fluctuations [8]. Using the CC-CE tool with the closed separation system eliminates these fluctuations. Several relevant papers are illustrated by this specific CC-CE technology. Its fully automated CC-CE operations are described in a currently available literature [9].

In fact, CC-CE was transferred while introducing the CC-CE chip [10]. The closed separation system on the CC-CE chip is benefiting: (a) various cross-sections for the separation channels, (b) short separation paths, (c) joining the electromigration techniques (CZE, ITP, etc.), (d) highly reproduced for the column-switching operations, (e) supporting for a relatively high sample volume, (f) concentration(s) of the analyte(s), (g) including for various sample clean-up (column-switching), etc.

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L-10

MICROSEPARATIONS AND MASS SPECTROMETRY COUPLING

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L-11

MICRO-SEPARATIONS NEED MICRO-LIGHT SOURCES: LEDS AND MICRO-LEDS AS LIGHT SOURCES OF MINIATURIZATION

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Abstract

Light emitting diodes (LEDs) are nowadays frequently used light sources in all areas of science and technology including chemical analysis, separation science, capillary electrophoresis (CE), capillary electrochromatography (CEC) and nano-capillary liquid chromatography (nano-LC). Their broad wavelength coverage from the infra-red and through the visible regions down to deep-UV and their outstanding properties, including low price, long lifetime and compatibility with miniaturisation, make them attractive light sources for miniaturised and portable instrumentation. In particular, their small size and low power consumption and heat emission allow LEDs to be located very close to the detection point, which is usually filled with liquid such as in a capillary or separation channel.

In this presentation, an overview of the state of the art in the field of the LED-based light sources will be given. Options for LED-based photometric and fluorimetric detection systems with focus on capillary detection in CE will be discussed and illustrated by selected examples. Photometric detection will be demonstrated with on-capillary detection in CE with a 255 nm deep-UV LED. The applicability of high power LEDs, such as the LUXEON, as an alternative light source for LIF instruments (LED-IF) will be demonstrated. A special attention will be paid to micro-LED arrays, their fabrication and their application as LED-IF light sources in CE. The advantages and disadvantages of the micro-LED-optical fibre coupling and alternatives will be critically discussed.

Acknowledgement

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L-12

TWENTY YEARS OF CONTINUOUS BED (MONOLITHIC) TECHNIQUES: UNSURPASSED FLEXIBILITY AND SUCCESS

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Abstract

The pioneering work on synthesis and application of non-particulate stationary phases for liquid chromatography was published in 20 years ago [1]. Numerous publications, growing interest and a number of non-particulate phase products released by several companies, such as CIM Disk (BIA Separations, Ljubljana, Slovenia), CB Silica plate (Conchrom, Bremen, Germany), SepraSorb (Sepragen, San Leandro, California), CIM Tube, CIM Disk (BIA Separations, Ljubljana, Slovenia), UNO (BioRad, Richmond, California), Swift (ISCO, Lincoln, Nebraska), Chromolith, Chromolith CapRod (Merck, Darmstadt, Germany), Monoliths (LC Packings, Amsterdam, The Netherlands), Onyx (Phenomenex, Torrence, California) illustrate an importance of this technique for chromatographic analysis and its maturity. The monolithic (continuous bed) stationary phases primarily synthesized as chromatographic media for biocompound separations are regarded as the fourth generation of biochromatographic stationary phases. Monoliths (continuous beds) are widely used in the HPLC and capillary format separations. They are also used in the chip format separations. Compatibility of the technique with the capillary format separations gained particular interest of the researchers and companies developing chromatographic columns for micro and nanoseparations (column I.D. >150 µm and I.D.<150 µm respectively). Three most important reasons can be mentioned. First is the simplicity of "packing" of the capillary, which is accomplished by drawing the solution of the continuous bed precursors into capillary using vacuum or moderate pressure and simultaneous synthesis of the stationary phase in situ. Second reason is no need of the supporting frits, installation of which in the particulate capillary column technologies requires certain experience and often causes problems of permeability or mechanical strength or bubble formation operating in CEC. The third reason for the popularity of the non-particulate stationary phase technologies is the flexibility of the technique. Due to the huge number of the monomers available, numerous chemistries can be applied to synthesize the monolithic columns and limitless functionalities can be embedded in the stationary phase during the single polymerization step or via reactive monomers attached in the next step by the covalent binding (polymer – analog reactions or graft – polymerization) to the surface of the non-particulate matrix.

In terms of continuous skeleton backbone chemical composition, the monoliths (continuous beds) may be classified as organic (acrylic, acrylamide, polystyrene, norbornene or mixed) and inorganic or mixed (organic and inorganic).

L-13

FABRICATION OF MICROCHIPS WITH CHROMATOGRAPHIC PACKINGS

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Abstract
There are only a few chip-based chromatographic systems compared to the chip-based capillary electrophoretic (CE) devices. Herein, we describe the design of a disposable and inexpensive microfluidic chip, fabricated from poly(dimethylsiloxane) (PDMS), incorporating chromatographic packings without the use of frits or permanent physical barriers, tapers or restrictors [1]. The packing of the chromatographic particles into the microfluidic channels is made possible by the hydrophobic nature and excellent elasticity of PDMS.
In our work both commercial reversed-phase (RP) chromatographic packing material and ground aerogel made in our department were used. The RP chromatographic material consisted of C18-modified, 5-10 □m diameter particles (beads). The aerogel is derived from a silicagel in which the liquid component of the gel has been replaced with a gas, thus the aerogels have a three-dimensional highly porous structure with pores just under 100 nm. Both chromatographic packings were proved to be suitable for efficient separations in chip.
The injection of a subnanoliter volume of sample into a microchip is a critical point of microfluidics. The most commonly used form of sample introduction in microchips is electrokinetic injection mainly due to its ease of use, however its limitations for quantitative determination is known. The chips including the high-flow resistance chromatographic packing spontaneously solved several injection problems well-known in microfluidics technology.
As a simple, external and universal detection, UV spectrophotometry was used in the disposable PDMS chips, although it suffers from the poor (concentration) detection sensitivity due to the short light path through the microfluidic channel, but it was suitable to easily monitor the separated components for testing the chips developed [2].

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L-14

POTENTIALITIES OF MICRO PREPARATIVE ISOTACHOPHORESIS AS A SAMPLE PRETREATMENT TOOL FOR MASS SPECTROMETRY

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Abstract
Pharmaceutical compounds, e.g., drugs and their metabolites, are reflecting for (ultra)trace concentration levels of biological samples (urine, serum). Such samples are challenging for various analysts as their matrices consist of several hundred constituents and still covered for very significant concentration levels. In addition, these span for various biological/chemical properties. Due to these, the separation techniques are required to be combined with the detection methods of adequate sensitivities and/or selectivities.
Techniques of capillary electrophoresis are operating for high efficiency separations and still using very low consumptions of the samples and the electrolyte solutions as well. Mass spectrometry detection provides an excellent selectivity while keeping sufficient detection sensitivity.
Cetirizine (antihistaminic substance), analyzed by direct injection electrospray mass spectrometry (DI-ESI MS) while present in different CE buffers, served as a model compound for the evaluation of electrospray efficiency for both the positive and negative ionization modes.
Micropreparative isotachophoresis (pITP) experiments for busereline (model analyte) with urine samples (model matrix) were performed in a hydrodynamically closed separation system to isolate busereline from a rest of the matrix components and concentrate for the analyte. An isolation procedure of the analyte was performed with the aid of a valve fractionation as based on our pITP apparatus while included for ITP discrete spacers as added to the sample. The isolated ITP fractions were lyofilized while followed by final mass spectrometric analyses. These were employed: (a) a direct injection mode, (b) UPLC/MS and (c) CE/MS combinations.
This work deals with different approaches for our analyte (a short peptide, busereline) as present in urine at very low concentration level by using pITP as followed by chromatographic and electrophoretic techniques coupled with mass spectrometry. MS and MS/MS spectra, obtained from the reconstructed fractions, proved both the pITP clean-up effect and its concentrating power for very low concentration levels of the analyte as present in the complex samples. In fact, this study shows high potentialities and compatibilities of pITP while regarding the sample pretreatment as preceeding for several modes of the mass spectrometry analysis.

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L-15

NANOTECHNOLOGIES IN BIOANALYTICAL CHEMISTRY

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Abstract

The typical properties of nanomaterials offer extraordinary possibilities to specially designed analytical systems. The combination of specific probes including immunofluorescent probes with highly fluorescent labels and an advanced optical instrumentation allows even the single molecule probing of individual cells. The application of highly stable Quantum dots in laser-induced fluorescence detection (LIF) and surface enhanced Raman scattering (SERS), are two examples of methods where the advantages of nanoparticles are taken. While in LIF, the fluorophor with high quantum efficiency must be conjugated with a high-affinity selector, an antibody, in SERS, the nanoparticles serve as centers where the electromagnetic energy of light is accumulated nonselectively in the form of surface plasmon and transferred to the adsorbed molecules of analyte to excite them. The objective of this presentation is to show the methods of the application and characterization of nanoparticles in analytical and diagnostic practice. Due to the broad excitation (350-500 nm) and narrow emission spectra (58nm), up to 4 probes excited by a single laser for parallel detection of several molecules and/or receptors in flow cytometry can be used. The examples of detection of important molecules and receptors in cells by high sensitivity fluorescence microscopy will be demonstrated. Namely, CD3 molecules connected with the TCR receptor of human lymphocytes and PCNA molecules in mouse embryo tissues. Another example of the preparation of detection probes is the nano CdTe probe for simultaneous electrochemical and fluorescence detection of oligonucleotides.

L-16

FABRICATION AND CHARACTERIZATION OF MICRO SOLID AMALGAM ELECTRODES (μSAE) FOR BIOANALYTICAL APPLICATIONS.

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Abstract

Electrochemistry represents one of the promising methods for detection of proteins, nucleic acids and their components in miniaturized systems. Practically all proteins (down to subnanomolar concentrations) produce well developed chronopotentiometric peak H [1] at hanging mercury drop and bare solid amalgam electrodes. Although the mercury electrodes have played an important role in the bio-analysis, development of new analytical tools based on nontoxic, environment-friendly materials retaining the electrochemical features of the mercury electrodes is of great interest. One of the promising materials for replacing liquid mercury is non-toxic solid amalgam. Such amalgam electrodes could be prepared in a microarray format allowing automation and integration with microfluidic devices.

In this work we have optimized microfabrication methods based on galvanic mercury amalgam formation on vacuum deposited thin films. Individual electrodes were formed on a nanolayer of a selected metal deposited on a glass surface and separated by a photolithographically patterned insulating layer. Geometry and composition of the resulting amalgam film were examined in detail using scanning electron microscopy (SEM) with energy-dispersive X-ray microanalysis (EDS). Quantitative analysis showed that amalgam is uniformly distributed all over the metal surface. The electrodes were electrochemically characterized with respect to their size, stability and sensitivity. Surface modification of the electrodes was tested for DNA, proteins and/or small molecule analysis. The microfabrication protocol provides electrodes with excellent reproducibility and sensitivity comparable to standard microelectrodes. Additionally, the new system provides easier and faster manipulation with much lower sample consumption. Further development of applications for routine protein and gene diagnostics on inexpensive disposable amalgam electrode array is under way.

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L-17

ELECTROCHEMICAL METHODS FOR MEASUREMENT OF REACTIVE OXIDIZING SPECIES

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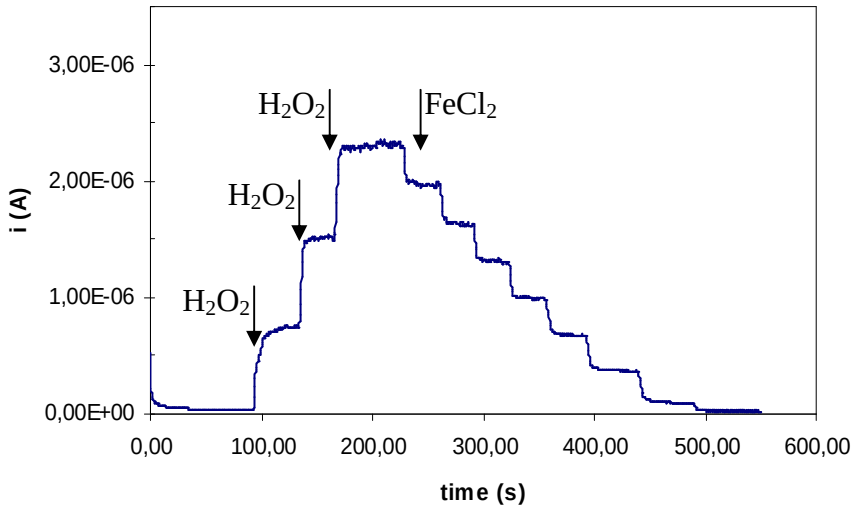
Abstract

The participation of reactive oxidizing species (ROS) in numerous physiological processes, including aging, signal transduction and some kind of immune functions is proved and their role is intensively investigated. The ROS induced oxidative stress is gaining growing attention in health care sciences owing to its involvements in development of a wide spectrum of diseases, such as dermatological, neuronal, immunological disorders.

The species of the ROS group are electro active that means they can be electrochemically oxidized, or reduced. Therefore for in vivo determination of their in situ, instantaneous concentration, electrochemical methods are the most promising. In our work development and improvements of selective methods for electrochemical detection of these molecules and radicals are attempted.

In this work to be presented here amperometric detection technique was employed with three electrode arrangement. We prepared size-exclusion layers by electro polymerization of m-phenylene-diamine monomer on the surface of working electrodes of different kinds to ensure selectivity. The conditions for this electro polymerization have been optimized. With this approach we succeeded to selectively detect very low concentration of H2O2 and NO in vitro.

Further experiments are in progress for development of methods capable of following concentration changes of ROS resulted by alcohol induced oxidative stress in vivo, in body fluids of anesthetized experimental animals.



Amperometric detetion of 10-30 μM H_2O_2 and the effect of $OH^{\cdot}$ production in Fenton reaction, (OD=1 mm Pt electrode, E=0.65 V in pH=7.4 PBS)

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L-18

CAPILLARY ISOELECTRIC FOCUSING COUPLED TO MASS SPECTROMETRY

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Abstract

The major problem in coupling isoelectric focusing to mass spectrometry arises from the disturbing presence of ampholytes, when the sample components enter MS. The „sandwich" injection protocol developed previously offers a unique opportunity for avoiding the entrance of the ampholytes into the MS analyzer, while the separation of the analytes is still satisfactory. The protocol allows a separate introduction of the ampholyte zone(s) and sample zone, but moreover the pH range of the ampholytes are not necessarily covering the pIs of the analytes. With this setup the sample components are migrating through the ampholytes, and leave the capillary end at different time (position) compared to the disturbing ampholyte components. The substituted amino-methyl-phenol dyes (p*I*: 5.3, 6.4, 6.6, 7.9, 10.4) and other analytes (p*I*: 2.7, 3.0, 3.5) are successfully separated with narrow or broad pH range ampholytes in uncoated capillary. By replacing the anolyte (phosphoric acid) and catholyte (sodium hydroxide) to volatile acid and base (formic acid and ammonium hydroxide) it is possible to separate the analytes at the beginning (or at the end) of the ampholyte zone, and detect them in the presence of low amount of ampholytes. The modell calculations show the system to be working and providing a successful approach for the separation of the pH gradient from the separated components. Practical experiments and simulations show that the original "sandwich" setup can be replaced by the simple consecutive injection of the ampholytes and samples (with either order).

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ABSTRACTS

POSTERS P-1 - P-46

P-01

ISOLATION OF PHARMACOLOGICALLY ACTIVE PROTEINS AFFECTING BLOOD COAGULATION FROM BULGARIAN VIPER VENOM (*VIPERA AMMODYTES MERIDIONALIS*)

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Abstract

The snake venom is a rich source of physiologically active compounds, most of which are associated with the toxicity of the venom (neurotoxins, myotoxins, hemotoxins, cardiotoxins, etc.). An interesting group of compounds with a potential pharmacological interest are those that affect the process of blood coagulation. Some of them are with procoagulant activity (serine proteases; potentially used in coagulation-factor deficiency treatment), anticoagulants (phospholipase A2 and metalloproteases), and proteins with fibrinolytic, fibrinogenolytic and thrombolytic activity.

The Eastern long-nosed viper *Vipera ammodytes meridionalis* populates mainly Bulgarian area and is recognized as one of the most venomous snakes in Europe. The venom has both proteolytic and neurotoxic components and contains hemotoxins with blood coagulant properties, similar and as powerful as in the crotalid venom. Other properties include anticoagulant effects, hemoconcentration and hemorrhage.

In the present study, an initial screening for the presence of proteins that affect blood coagulation is performed. The crude snake venom was separated using column chromatography on SP-Sephadex and the collected fractions were tested for proteolytic and anticoagulant activities. Then further FPLC separation on Mono S column was applied. The subfractions collected were characterized using SDS-PAGE, enzyme activity, and coagulation tests. The enzyme activity of the homogenous proteins was additionally studied and detailed hemostaseological characterization was completed.

As a result of the screening study performed, some new proteins affecting blood coagulation process were isolated. All of them exhibit proteolytic activity which was inhibited either from EDTA , presenting evidence of metalloproteinases, or from PMSF, suggesting serine type proteases.

Acknowledgement

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P-02

ANION-EFFECT ON THE SPECTROSCOPIC PROPRETIES OF MERCURY CONTAINING FLUORESCEIN DERIVATIVES

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Abstract

Ion selective electrochemical or optical sensors are often used in a determination of anions concentration in environmental or food samples [1]. The typical dynamic range of the sensors is around in the 0.01-100 mM range. However, there are some applications, where a lower limit of quantification is required. The sensing principle of optical anion sensors are mainly based on the luminescence quenching of indicators by the anion of interest. Thus, indicators with permanent positive charge are often used for the determination of anions [2].

In this work commercially available (merbromin and 4',5'-bis(acetoxymercury)-fluorescein) and newly synthesized (fluorescein-Hg_2-nitrate and fluorescein-octylester-Hg_2 nitrate salts) mercury derivatives of fluorescein were investigated as promising sensing materials for low level chloride ion determination. Spectrophotometric, fluorescence intensity and phosphorescence decay time measurements were performed in ethanol/water mixtures in the absence and in the presence of anions. The stochiometry and the sensitivity toward anions were determined. Interestingly the fluorescence intensity was decreasing, while the phosphorescence intensity was enhanced by adding increasing amount of chloride or thiocyanate ions. The indicators were also immobilized and tested in polymeric nanobeads [3].

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P-03

USING DISCRETE SPACERS AS ENHANCING THE RESOLUTION IN CAPILLARY ZONE ELECTROPHORESIS WITH COUPLED BY CAPILLARY ISOTACHOPHORESIS

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Abstract

Capillary zone electrophoresis (CZE) with isotachophoresis (ITP), as based for a column-coupling technique (ITP-CZE), was investigated to apply discrete spacers (DSs) as loadable into the samples of complex ionic mixtures.

Appropriately chosen DSs were performed for the ITP zones and, in parallel, as loaded the sample constituents to the ITP boundary layers (reflecting for the ITP steady state). For obvious reasons the leading ions were transported outside from the separation system. Subsequently, the ITP stage was followed for the CZE stage to destack the sample constituents.

In our study were included ITP-CZE (without using the DSs) and ITP(DS)-CZE (with the DSs) while operated for computer simulations (SIMUL, B.Gaš et al.). For example, these simulations were taken for 40 model analytes with and without three DSs. In fact, these model analytes enhanced the resolutions for ITP(DS)-CZE, especially, when compared for ITP-CZE.

Our experiments for ITP(DS)-CZE and ITP-CZE, in the cationic mode, were carried out for complex human urine samples. Importantly, these experiments were significantly documented to enhance the resolutions of ITP(DS)-CZE while compared for ITP-CZE.

In fact, using the simulation and experimental investigations, increased the use for the second separation dimensions by ITP(DS)-CZE. Still, these ITP(DS)-CZE simulations, as operating for different model analytes, showed some logical restrictions for these second dimensions.

This work was supported by grants from the Slovak Grant Agency for Science (VEGA 1/0882/09) and the Slovak Research and Development Agency (VVCE-0070-07).

P-04

EXAMINATION OF THE PROTEIN PROFILE OF *CANDIDA ALBICANS*: EFFECT OF FUSED MANNICH KETONS

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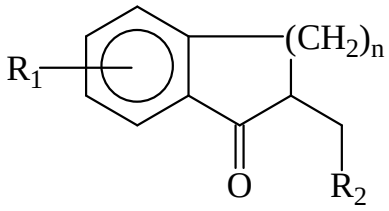
Abstract

The incidence of *Candida* infections has been growing during the last few decades. The resistance against well-known antifungal agents, like azoles, also increased.
Mannich ketones are a large family of compounds with known cytotoxic, antibacterial and antifungal effect. The aim of this study was to examine a larger group of fused Mannich ketones and determine their effect on the protein composition of *C. albicans*.
The test compounds were prepared using the classical acid-catalysed Mannich reaction starting from fused bicyclic ketones as 1-indanones and 1-tetralones.



n= 1-3 ; R1= H, 5-OCH_3 , etc. ; R2= 1-piperidyl,etc.

The protein profile of *C. albicans* was examined by a capillary electrophoresis based microfluidic system. The commercially available Agilent 2100 Bioanalyzer was utilized with the Protein 80 and 230 Kit. We tried to improve the technique for our aims and amend quantification.
Some of the compounds studied were found to induce alteration on the protein-profile of *C. albicans*.

P-05

APPLICATION OF FIVE-PARAMETERS WEIBULL EQUATION FOR DETERMINATION AN AVERAGE CONCENTRATION OF METHANE IN BIOGAS FROM CORN SILAGE IN THE PERIODIC METHANE FERMENTATION PROCESS

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Abstract

The methanization process of several agricultural and organic municipal solid waste is most popular method of renewable energy production [1]. The concentration of methane in produced biogas was measured with used gasses analyser GAS DATA GFM 430 during the laboratory periodic fermentation processes. The hrydraulic retention time (HRT) of singe experiment amount 120 days [1]. The concentration of methane in produced biogas was expressed by the curve CCH_4 [%] = *f*(HRT [d]) and presented in Fig. 1.

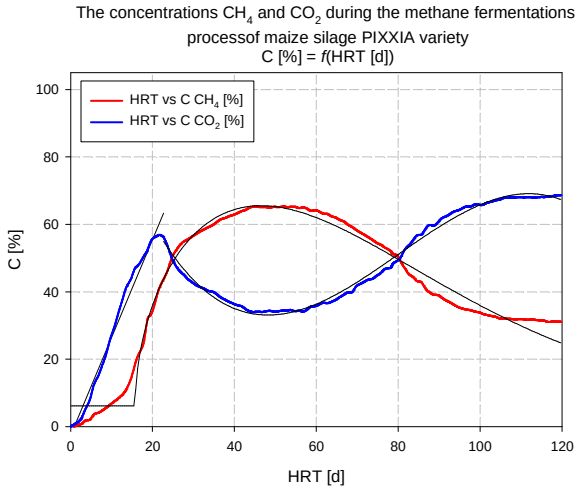

Fig. 1. Relationship between the concentrations of CH_4, CO_2 and HRT in produced biogas during the anaerobic fermentation of maize silage PIXXIA variety

Red curve shows variation of methane concentration in biogas. It's describe by five-parameters Weibull equation. Comparison of areas under the curves gives information about average concentration of methane. This information can be use like parameter from continuous methane fermentation process.

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P-06

EVALUATION OF THE CARBON TO METHANE CONVERSION COEFFICIENT DURING THE ANAEROBIC FERMENTATION PROCESS OF SELECTED MAIZE SILAGES

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Abstract

The coefficient of carbon to methane conversion (β) is the main parameter which determination of efficiency the methane fermentation process. The methan fermentation progress was followed by measuring the volume and composition of gaseous product evolved. The following methods were applied: elemental analysis (EA), five-parameters Weibull equation [1] and computational methods. In the experiment ten species of maize was used. The value of beta coefficient was described by the equation No. 1 [2].

$$\beta_{C \rightarrow CH_4,120} = \frac{nC_{CH_{4eq}}}{nC_{EA}} \qquad (1)$$

where: nCH_{4eq} – mol number of carbon in methane (calculation with use Weibull equation), nC_{EA} – mol number of carbon in maize silage (determination by EA)

The β coefficient ranged from 26.8±0.7% to 47.9±1.1%. The largest of β value for PIXXIA species, the smallest for MAXXIS species were determined.

An information about quantity of carbon conversion coefficient provide guidance for methane fermentation processes which were used for production of biogas. The carbon to methane conversion coefficient can be use like a reliability of information about bioavailability during the production of energy with use methane fermentation processes.

The authors wish to thank Ministry of Science and Higher Education for the financial support grant No. 3313/B/P01/2009/36 31 (607-Ch).

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P-07

DIRECT DETERMINATION OF ORGANIC ACIDS IN CELEBROSPINAL FLUID BY ZONE ELECTROPHORESIS AS USED A COLUMN-COUPLING CHIP TECHNOLOGY

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Abstract

Organic acids, accumulated in cerebrospinal fluid (CSF), are provided useful information for metabolic diseases. In fact, such a link was supported to benefit for capillary electrophoresis (CE) as clearly documented by a recent paper [1]. In addition, this paper is showing a direct determination of organic acids in CSF by CZE. Apparently, this is simplifying for the analytical procedure.

We developed a CZE procedure for the determination of organic acids (oxalate, citrate, glycolate, lactate, 2- and 3- hydroxybutyrate) in CSF samples as based on the column-coupling chip (CC-chip) and integrated with the conductivity detection. It should be stressed the CC-chip operates for a hydrodynamically closed separation system and, in addition, suppressing for the electroosmotic flow.

We were favoring a single-column CZE separation, joining a pair of the separation channels on the chip, as proposed for the background electrolyte solution (pH = 5.5). It should be stressed as in our CZE separation runs was operated, when needed, for the column-switching regime (indicated by the CZE-CZE separations).

For the CZE-CZE separation runs we were including fluently for the sample clean up. For example, to remove a dominating part of chloride as migrating outside from the separation system (an extremely high concentration of chloride in CSF) while determinable for oxalate.

Our CC-chip is loaded a relatively high sample volume (900 nl) and this made possible to reach 0.1-2.1 μmol/l concentrations of organic acids as corresponding to the limits of detection. Highly reproducible migration times (up to 0.8% RSD) and the peak areas of the analytes (ca. 7% RSD) were found for long-term time intervals as including for model and CSF samples. The samples were diluted ca. 100-fold while directly analyzed without any other sample pretreatment. In general, such a simple analytical procedure is taken for about 400 seconds.

This work was supported by grants from the Slovak Grant Agency for Science (VEGA 1/0672/09 and 1/0882/09), the Slovak Research and Development Agency (VVCE-0070-07) and, in part, supported by Merck (Darmstadt, Germany).

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P-08

MASS SPECTROMETRIC ANALYSES OF LIPOPOLYSACCHARIDES EXTRACTED FROM SHIGELLA SONNEI ROUGH-TYPE MUTANT STRAINS

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Abstract

Endotoxic lipopolysaccharides (LPSs) are the major components of the envelope of almost all Gram-negative bacteria. These molecules, responsible for both, the advantageous and the harmful biological activities of these micro-organisms, are highly immunogenic and directly involved in numerous bacterial diseases in humans such as Gram-negative sepsis. The structural study of intact LPSs by mass spectrometry contributes to the understanding of processes related to their physiological effects and bacterial pathogenesis.

Typically, the S-type (smooth) LPSs usually constitute a hydrophilic moiety consisting of an O-specific chain and the core oligosaccharide, covalently linked to a lipophilic moiety (lipid A) that anchors LPS to the outer membrane. R-type (rough) LPSs do not possess any O-specific polysaccharide and sometimes lack portions of the core, as well. Lipid A (having defined comformation and fatty-acyl content) constitutes the endotoxic principle of the LPS molecule, expressing all the pathophysiological effects known to be induced by these molecules.

In this poster, the structural variations in the R-type endotoxins of *Shigella sonnei* mutant strains are discussed. MALDI-TOF MS and MS/MS investigations were performed. Spontaneous mutation of *S. sonnei* phase I strain (a pathogenic member of the family *Enterobacteriaceae*) leads to the non-pathogenic *R*-type, *S. sonnei* phase II (*4303*), which lacks the O-antigen. From this strain, a series of *R*-type mutants were obtained by ethyl-methyl-sulfonate induced mutagenesis. *S. sonnei R41*, *562H* and *4350* strains were isolated and their LPS content, as well as the LPS of *4303* "maternal" strain, were analyzed. A lipid A moiety was obtained by mild acid hydrolysis from LPS of *S. sonnei R41*.

The detailed evaluation of the mass spectra indicates heterogeneity in the fatty acid composition and variability in phosphorylation stage. The oligosaccharide cores of the rough mutants contain two 3-deoxy-D-manno-2-octulosonic acid (Kdo) units. The LPSs of the isogenic rough mutants *S. sonnei 4350 562H, R41* and *4303* are formed in a step-like manner containing 0, 1, 2 and 3 heptoses, respectively, in the inner core region. The outer core region of the LPS from the *4303* mutant contains also hexoses.

The work was supported by the grants GVOP-3.2.1-0168 and OTKA-NKTH-NI-68863.

P-09

TO ASSESS THE ACTUAL HUMAN EXPOSURE TO PAHS GENERATED FROM INDUSTRIAL , TRAFFIC AND RURAL SETTINGS IN SLOVAKIA

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are products of incomplete combustion or pyrolysis of various organic materials. Their ubiquity in the environment leads to measurable levels of exposure. However, the exposure varies strongly between different regions in Europe. Some PAHs with four or more rings are suspected to be human carcinogens. Therefore the occupational and/or environmental exposure to PAHs may cause a significant health risk. Since PAHs are always present in complex mixtures consisting of up to 100 or more different PAHs the related exposure must be taken as a combined exposure in any case.

Traditionally, the human exposure to PAHs is assessed by air quality measurements. In most studies, the determination of 16 PAHs in air (according to US EPA) is used as an indicator of airborne PAHs.

It is hypothesized that biomarkers of PAH exposure may predict the lung cancer risk of individuals. Therefore, biological monitoring is being used to evaluate the human health burden associated with exposure to PAHs. Until now, only very limited biological monitoring data on actual exposure to PAHs in Slovakia has been reported.

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P-10

APPLICATION OF ARTIFICIAL GEL ANTIBODIES FOR DETECTION AND QUANTIFICATION OF BIOMARKERS IN CLINICAL SAMPLES

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Abstract

Artificial gel antibodies can be synthesized by a universal method against biopolymers, for instance proteins, and bioparticles, for instance viruses and cells. These artificial gel antibodies show high selectivity and are easy to prepare. The selectivity and stability of these antibodies are much higher than for the native protein antibodies.

We have designed a calibration curve, based on the light absorption of the stained protein, adsorbed to gel antibodies. The calibration curve is used for rapid determination of the concentration of the "antigen" in a sample solution, for instance blood serum. One potential application of the artificial gel antibodies is to "fish out" a biomarker for a specific disease from serum for diagnosis and prognosis of this disease. The calibration curve we will present can be used for the determination of the concentration of the protein biomarker down to 20 µg protein/ml in the body fluid.

In the present work we have synthesized artificial gel antibodies against human albumin with the aim to develop a simple and rapid procedure to measure the concentration of this protein in samples of clinical interest. The procedure, based on the design of a standard curve, was applied on a quantitative analysis of albumin in human plasma and cerebrospinal fluid (CSF). We found that our technique permitted detection of albumin in these body fluids with high precision and that the concentration of this protein was significantly enhanced in CSF from patients with amyotrophic lateral sclerosis (ALS), compared to control samples.

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P-11

ORTHOGONAL OFF-LINE COMBINATION OF RP-HPLC AND SEC FOR ANALYSIS AND CHARACTERIZATION OF SELECTED ENVIRO-BIOPOLYMERS

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Abstract
Humic substances (HS) belong to the most spread environmental biopolymers and they have direct influence to various processes playing significant role in an environment. Relatively few works solve problems of off-line (alternatively on-line) combination of two or more chromatographic methods based on different principles and their application to the characterization of HS. This fact is surprising, because adoption of this approach could lead e.g. to more complex insight to their behavior in different chromatographic systems.
All recently used single separation techniques have definite limitations in terms of their selectivity and separation range, what leads to the necessity of multi-stage separation procedures for samples, which contain a wide variety of different components. Current praxis requires fast and rugged analytical methods which can provide comprehensive information about the complicated row materials. This, together with the need of numerous HS samples of various origin analyses dictates the necessity of development automated complex separation procedures with minimal sample pretreatment. The use of on-line multidimensional chromatographic techniques is a logical solution of the requirement. Multidimensional chromatography has proven to be useful for the analysis of complex samples such as humic substances.
The aim of this work was an introductory study of off-line combination RP-HPLC and SEC, both with mobile phases containing dimethylformamide. With respect to the non-common approach we focused to evaluation of its potential to create orthogonal, i.e. on different separation principles working two dimensional comprehensive separation methods with a compatible mobile phases.
Obtained result indicate, that such methods can be combined in a compact, automatic, orthogonal separation system for characterization of such complicated natural substances as are examined humic acids. By this means we can get more information about the HS character.

Acknowledgments
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P-12

FRACTIONATION OF ENVIRONMENTAL BIOPOLYMERS BY IMMOBILIZED ALUMINIUM(III) METAL ION AFFINITY CHROMATOGRAPHY

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Abstract
Immobilized metal affinity chromatography (IMAC) is gaining widespread popularity as an effective tool for the separation and characterization of a variety of biological macromolecules [1]. IMAC is based on the ability of separated ligands in solution to form coordination complexes with metal ions that are fixed to the immobilized multi-dentate ligands attached to the surface of stationary phase within a column. The prevailing separation mechanism is an additional complexation of structurally predisposed analytes to the bound metal ions that have not saturated coordination number. IMAC is most commonly used in biochemistry, molecular biology and related branches of science for isolation of ligands that bind soft metal ions (e.g. Zn, Cu, Ni), e.g. proteins, peptides etc. [2].
In the present work, IMAC was developed for the fractionation of biopolymers, specifically humic acids (HA). In our case, Iontosorb Salicyl was used as the chelating resin containing salicylic acid bound via azo group in side chains of modified bead-form cellulose. Sorption characteristics of Al(III) ions at this chelating sorbent were determined at different pH values. Simple photometric detection at 590 nm was used for monitoring aluminium as SPADNS-Al(III) chelate in eluent during breakthrough experiments. Resulting sorption capacity is highest at pH 5.5 giving value 36 µmol of aluminium per 1 g of the sorbent.
Next study was focused on the fractionation of HA by Al-loaded Salicyl IMAC technique by the effect of mobile phase various pH values (3.9, 6.7 and 8.9, resp.) Finally, IMAC technique was used for the fractionation HA applying a buffer-based pH gradient (between pH 8.9 and 2.0) for their gradual elution. The HA fractions retained by the Al(III)-IMAC were eluted with mobile phase of decreasing pH value, and the fractions collected were characterized by UV–VIS detection. Based on the results obtained, IMAC appears to be a promising tool for humic substances separation.

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P-13

ANALYSIS OF SOME COMPONENTS EXTRACTED FROM *SCILLA MARITIMA* GROWING IN THE NORTH OF ALGERIA

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Abstract

The aim of this work had been consisted of extraction, analysis and characterization of some heterosides from the *Scilla* (*Urginea Maritima*) which is growing in the middle north of Algeria (Kabylia). The dried powder had been submitted then to toxicological and bactericidal analysis.

The choice of solvent had led to the following mixtures: water; mixture EtOH-water (80 : 20v/v)], MeOH-water (80 : 20v/v), EtOH (8 spots), MeOH (10 spots), acetone (10 spots).

For the TLC, we used Silicagel 60 F254+366 Merck plates, chloroform/ methanol/ dimethylformamide (80 :19 :1, v/v/v) as mobile phase and Carr-Price reagent under 366 nm.

The HPLC analysis had been led under the following conditions: JASCO PU 1580 with JASCO UV-1570 (UV/VIS Detector); Discovery C18 (250mmX4.6mm, 5µm column); acetonitrile / water (30 :70, v/v), rate: 0,7 ml/min. λ= 280 nm.

Test results of acute toxicity are shown in the table and in the figure

Dosis mg/Kg	Number of deaths				Symptoms
	24 h	48 h	72h	totale	
125.3	0	0	0	0	No phenomena of intoxication
150.4	0	0	0	0	Weakness
180.4	0	0	1	1	Convulsions
216.5	2	0	1	4	Tono-clonic convulsions, ataxia. tremors
260	3	0	1	4	

Calculation of LD50 by the method of Miller and Tainter allowed to find that : DL_{50} = 213 ± 22 mg/Kg., This result showed the effect of the rat poison dry.

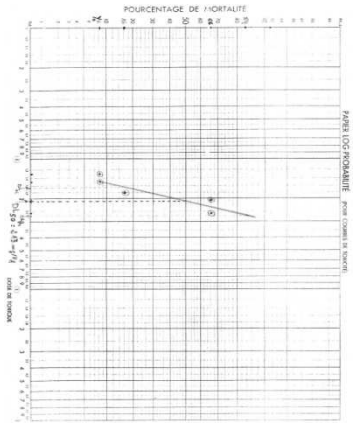

Fig.1. Dose-mortality relation of the dry

P-14

CHROMATOGRAPHIC (RP-HPLC) ANALYSIS OF CHOLESTEROL AND SELECTED PHYTOSTEROLS

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Abstract
Plant sterols (phytosterols) are bioactive components found in all vegetable foods; they resemble cholesterol in vertebrates in term of both function and structure. In the group of the most abundant phytostelors belong β-sitosterol, campesterol and stigmasterol. Phytosterols shows potency in decreasing the levels of low-density lipoprotein (LDL) cholesterol in the serum and thus in protecting against cardiovascular diseases [1].
Current methods for the analysis of cholesterol (single, esterified, sulphated), as well as other sterols and lipid classes include TLC, SPE, GC, HPLC and SFC [2], among these HPLC operates under milder column temperature and non-destructive detection conditions. Additionally, utilizing RP-HPLC employs less volatile polar organic solvents in water and offers ready equilibration between both phases. The selectivity of stationary phase for sterols differs from molecular size and the number of double bonds [2, 3].
In this study, HPLC separation of mentioned compounds was optimized for their fraction collection and subsequent MS determination using Luna C18 (250x3 mm, 5 μm), Luna C8 (250x4.6 mm, 5 μm) and Luna C8 (150x4.6 mm, 3 μm) Phenomenex columns. Experiments were performed on HPLC system 10AVP with DAD; wavelength was set to 206 nm. . Acetonitrile, methanol, or their aqueous solutions, with/without addition of some less polar organic modifiers such as isopropanol or THF, were examined as a mobile phase composition. The influence of temperature (25-40 °C) and flow rate (0.5-1.0 $ml.min^{-1}$) were also investigated.
Luna C8 (150x4.6 mm, 3 μm) column, mobile phase MeOH/water (97:10), flow rate: 0.7 $ml.min^{-1}$ and temperature 25 °C under isocratic mode provide appropriate separation for off-line HPLC-MS analysis of cholesterol, β-sitosterol and stigmasterol. Sterols were characterized using MALDI MS with silver nanoparticles as matrix.

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Acknowledgments
We gratefully acknowledge Czech Science Foundation, grant No. 203/09/1025 and Ministry of Education, Youth and Sports of the Czech Republic, grants No. MSM0221622415 and LC06035.

P-15

EVALUATION OF THE EQUIVALENCE OF HPLC METHOD AND THE STANDARD SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF CREATININE

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Abstract

In laboratory practice sometimes is necessary to confront the results from analysis achieved by established method with another method, which is based on different principle. This task is simple, if the character of the work allows the comparison in the same concentration level. Demanding is to compare the methods in the whole range of performed analysis. This work describes evaluation process of newly established HPLC method and its comparison with standard spectrophotometric method for creatinine determination in urine. Evaluation of the methods was done on the basis of statistical analysis. Results showed that the newly established method gives statistically equivalent results as the standard spectrophotometer method and therefore the new method can fully replace it. The advantage of the HPLC method is its simplicity and possibility of synchronous determination of several different metabolites in urine sample.

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Acknowledgement

Research was supported by the project VEGA 1/4370/07

P-16

A DIRECT TRACE DETERMINATION OF GLYPHOSATE IN DRINKING WATER USING COLUMN-COUPLING ELECTROPHORESIS CHIP WITH CONDUCTIVITY DETECTION

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Abstract

Our study was developed an analytical electrophoresis procedure while introducing a trace based determination of Glyphosate [N-(phosphonomethyl)glycine] as regarding drinking waters. In fact, it was using capillary zone electrophoresis (CZE) with isotachophoresis (ITP) sample pretreatment as provided a full advantage of the column-coupling (CC) electrophoresis chip. This CC chip, a pair of the separation channels with the contact conductivity detectors, was suppressed for the hydrodynamic and electroosmotic transports in the ITP-CZE run. In addition, the sample injection channel on the CC chip was loaded either a 0.9 µl volume or a 9.9µl volume (as intended, especially, for a trace analysis).

For the ITP stage, a 4.5 µl volume of the ITP separation channel, was filled the electrolyte solution at pH=3.2. In fact, this stage was employed (i) to separate the sample constituents isotachophoretically and (ii) to transport electrophoretically the matrix constituents outside from the ITP-CZE separation system. Such an effective sample clean up was switched to transfer glyphosate to the CZE stage (a 4.3 µl volume of the CZE channel as filled-up with the background electrolyte solution at pH= 6.1).

A 2.7 µg/l concentration of glyphosate in drinking waters was estimated for the limit of detection (LOD) as loaded a 9.9 µl volume on the CC chip. This LOD value for glyphosate is much more lower when compared for the maximum contaminant level (MCL, a 700 µg/l concentration of glyphosate as reflecting for the National Primary Drinking Water Standards by the US EPA).

For the short- and long-term repeatabilities were found the migration times of glyphosate (0.1-3.5 % RSD) and the quantitation parameters (0.2-6.9 % RSD as expressed via the peak areas). Drinking water samples, as spiked by glyphosate at 10-100 µg/l concentrations, led to 99-119 % analyte recoveries. In the ITP-CZE determination of glyphosate in drinking water samples, diluted and/or degassed the samples before their loading on the chip, could be stated as a direct sample injection procedure while using the CC chip. A total analysis time of about 10 minutes could be considered as a time benefit for the ITP-CZE procedure on the CC chip.

This work was supported by grants from the Slovak Grant Agency for Science (VEGA 1/0672/09 and 1/0882/09), the Slovak Research and Development Agency (VVCE-0070-07), Comenius University (UK/307/2009) and, in part, supported by Merck (Darmstadt, Germany).

P-17

SEASONAL VARIATION IN THE COMPOSITION AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL OF *ROSMARINUS OFFICINALIS* FROM PAKISTAN

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Abstract

The essential oil from *Rosmarinus officinalis* leaves harvested from a same locality during summer and winter was compared in terms of yield, chemical composition and biological activities. The essential oil contents from the leaves of *R. officinalis* were 1.05 and 0.93 g 100g-1 from the summer and winter crops, respectively. The GC and GC-MS analysis revealed that the principal components determined in R. officinalis essential oils were 1,8-cineol (28.5 and 37.1%), camphor (15.9 and 15.8%), α-pinene (10.7 and 11.4%) from the summer and winter crop samples, respectively. Major fluctuations were observed in the contents of 1,8-cineol (29.2-38.5%), borneol (3.25-6.33%) and α-terpineol (traces to 2.30%) with respect to different seasons. The modified resazurin microtitre-plate assay was used to evaluate the minimum inhibitory concentration (MIC), while the antioxidant activity was evaluated by the reduction of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) and measuring percent inhibition of peroxidation in linoleic acid system. The antiproliferative activity was tested on breast cancer (MCF-7) and prostate cancer (LNCaP) cell lines using the MTT assay. *Rosmarinus officinalis* essential oil from the summer crop exhibited best antimicrobial and antiproliferative activities while that from the winter crop showed better antioxidant activity. The biological activities of the tested oils varied significantly ($p < 0.05$) with respect to the seasonal changes.

P-18

OCCURRENCE OF RISK ELEMENTS Cd AND Pb IN SMALL TERRESTRIAL MAMMALS FROM UPPER NITRA REGION

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Abstract

The content of accumulative metals cadmium and lead was investigated in fresh parenchymatosis organs (liver and kidney) of small terrestrial mammals (*Apodemus sylvaticus, Micromys minutus, Sorex araneus* and *Clethrionomys glareolus*) from the area of Upper Nitra region. ET-AAS metod was used for analysis of total content of these risk elements.

The mean content of Cd in liver and kidney was 0.093 $mg.kg^{-1}$ and 0.048 $mg.kg^{-1}$, respectively. Mean accumulation of Pb in the fresh tissues was 0.714 $mg.kg^{-1}$ (liver) and 0.024 $mg.kg^{-1}$ (kidney). The highest contents of the metals were found on locality Koš – near midden, where the accumulation of Pb in liver achieved value 4.86 $mg.kg^{-1}$. In 15 cases content of Cd or Pb exceeded limits set for wild animals (game).

Acknowledgement

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P-19

VALIDATION OF HPLC METHOD FOR QUANTITATION KETOPROFENE IN KETOPROFENID SUPPOSITORY

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Abstract

A few published works report about development of a validated method for ketoprofen [1,2].

The aim of this work had consisted of quantitation Ketoprofene in a 100 mg Profenid® suppository by a validated HPLC and UV/Vis. method.

The HPLC separation and proportioning was performed on a µBondarpack C I8 column (10µm); 125 A°; using methanol (99.8%) as a mobile phase. The analyte was monitored at 254 nm.

The average coefficients of variation were: CV= 0,2 and 0,1 for the standard and the suppository solutions respectively (repeatability), and CV= 0,3 and 0,2 (reproducibility)

The coefficients of correlation of the linear calibration were 0.9990 and 0.9966 respectively

For the UV method, the coefficients of correlation were 0.9997 and 0.9988 for the standard and suppository solutions respectively.

The average coefficients of variation of within-and between-day were :

0.22 and 0.20 for the standard solution and the suppository solution.

The Detection limit for this active ingredient range was found to be 2.5×10^{-4} g/ml.

The results obtained by HPLC show a very good correlation with those obtained by U.V visible.

The proposed method is sensitive, reproducible and very suitable for Ketoprofen determination in pharmacokinetic studies.

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P-20

MASS TRANSFER ON SUPERFICIALLY POROUS AND TOTALLY POROUS REVERSED PHASES IN HPLC

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Abstract

In the past few years several stationary phases were developed, which increase the velocity of the HPLC separations without decreasing efficiency.

Generally used HPLC stationary phase is totally porous particle. A mobile phase percolates through the porous particles, and the sample molecules get across in the pores stagnant mobile phase, where they get into an interaction with the stationary phase. The sample molecules progress with diffusion inside the pore, or diffusion necessary to the leaving of the pores. The sample is exposed to a number of mass transfer resistances in the course of the chromatographic process, and these influence that of the peak edge significantly. With the definition of the mass transfer kinetics, the effect of the geometry of stationary phases onto the peak shapes can be studied.

There are a number of sources for mass transfer resistance in the stationary phase particles: the axial dispersion (D_L), the external mass transfer resistance (k_{ext}), intraparticle diffusion (D_p) and the adsorption-desorption kinetics [1]. The definition of the mass transfer parameters are used plate height equations. The general rate model is a most detailed chromatographic model, which takes into consideration all of the sources of the mass transfer resistances. The van Deemter equation is an empirical plate height equation, which is widely applied, and considers the mass transfer phenomena.

In this work we determined and present the mass transfer coefficients of human insulin on the two different phases. Two kinds of stationary phases are examined: totally porous SunFire C_{18} and Halo with a porous shell C_{18} [2].

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P-21

ANALYSIS OF TRAJECTORY OF MAGNETIC PARTICLES IN MAGNETIC FIELDS FOR CELL SEPARATION AND TRANSFECTION

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Abstract

Novel systems in the research field of magnetic cell, drug or gene targeting using principle of magnetic separation by high gradient magnetic field were proposed and theoretical analyzed. System based on four electric wires with high electric current drives magnetic field with high intensity and gradient [1]. Magnetic field was assigned analytically from ideal wire quadrupole model. By analysis of magnetic nanoparticles as well as microparticles trajectories determined by numerical solution of ordinary differential equations describing their movement in viscous ambient and magnetic field, emerging from model based on selfdemagnetization and magnetic saturation of magnetite particles (Fe_3O_4) [2], we have recognized that mean capture time is sufficiently short, strongly depends on size of used magnetic particles (with increasing radius descends), and therefore biomedical application of such system for separation of cells with bonded magnetic microparticles as well as consecutive with magnetic field facilitated transfection by attracting target compounds with bonded magnetic nanoparticles is possible. Application of magnetic field alone won't be apparently sufficient for cell membrane transfection but at all events it will increase its probability by targeting of effective compounds on cell surface. We have also used similar methodology for cylindrical Halbach array, which produces magnetic field with smaller gradient and consequently mean capture times of particles were longer. For magnetic field modeling was used finite element method.

For the design of a novel potential system for magnetic separation in micrometric dimensions field with high gradient in vicinity of dots of strong ferromagnetic material (supermalloy; with radius 5 μm) in originally homogeneous field (magnetic flux density 0.46 T) was also analyzed. It was determined effective reach radius of this system in the case of nanoparticles (with radius 50 nm) in micrometers. Even in this case magnetic field has meaning for targeting effective compounds with bonded magnetic nanoparticles on surface with culture of target cells [3], whereby for transfection of cells the method can be combined with electroporation [4].

This work was supported by European Union grant "Magselectofection" (contract No. 019038).

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P-22

ZONE ELECTROPHORESIS SEPARATIONS OF ALIPHATIC AND AROMATIC AMINES ON A POLYMETHYLMETHACRYLATE CHIP

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Abstract

This study deals with zone electrophoresis (ZE) separations of thirteen aliphatic (ammonia, isopropylamine, 2-methoxyethylamine, 4-aminobutyric acid, butylamine, ethanolamine, N,N-diethyletylenediamine) and aromatic (benzylamine, 4-fluorobenzylamine, 4-methoxybenzylamine, 2-phenyloxyethylamine, 3-aminomethylpyridine, 3-aminobenzimidazol) amines on a poly[methylmethacrylate] (PMMA) chip with conductivity detection. From methodological point of view, this work was focused on the study of adsorption processes on the PMMA chip. Amines together with other constituents, e.g. peptides and proteins, have a strong tendency to adsorb onto the inner walls of electrophoresis separation systems (capillaries, chip channels). Interactions between these constituents and separation surface can cause changes in their migration and quantitative characteristics during electrophoresis separations. Therefore a modification of inner surface of the separation system is required to suppress adsorption of the sample constituents [1].

The surface of the PMMA chip was modified by dynamic coating to prevent an adsorption of studied amines onto the walls of chip channels. Surface modifiers, e.g. aliphatic oligoamines (diethylenetriamine or triethylenetetramine, TETA) were added to the electrolytes which filled the chip channels. These modifiers showed a higher affinity to interact with function groups on the surface of the chip channels than the analytes in model samples. An impact of the modifier concentration on the peak profiles and resolutions of the studied amines has been monitored. ZE experiments were realized in a cationic mode in acetate and propionate carrier electrolyte. An addition of a 100 μmol/l concentration of TETA (surface modifier) and a 25 mmol/l concentration of 18-crown-6-ether (complexing agent) in propionate carrier electrolyte has been used for the ZE separation and resolution of the studied amines. Dynamic modification of the surface of PMMA chip was very effective in reaching fast (ca. 10 minutes), sensitive (0.2-0.5 μmol/l concentration limits of detection) and reproducible (0.2-6.3% RSD of the peaks areas) ZE determinations of the studied amines.

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P-23

THE EFFECT OF COMPLEX FORMATION OF THIACALIX[4]ARENE WITH AMINO ACIDS ON THE TRANSITION THERMODYNAMICS AND KINETICS OF BOVINE SERUM ALBUMIN

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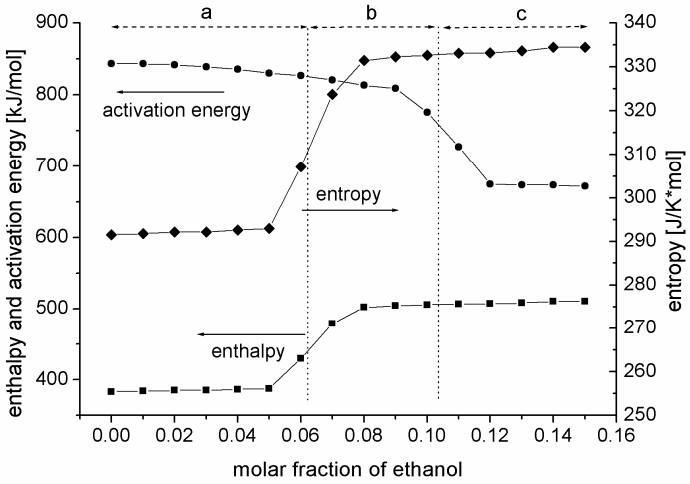
Abstract

Complexation ability of water-soluble tiacalix[4]arene-tetrasulfonate towards three aromatic amino acids (Phenylalanine, Tyrosine and Tryptophane) was studied in water-ethanol mixtures by photoluminescence (PL) method as a function of the ethanol content of the bulk solutions. Job's method followed by the application of the van't Hoff theory was used to determine the thermodynamic parameters of the molecular association. Results show quite different thermodynamics of formation of calixarene-amino acid complexes at low and higher ethanol content of the solutions. The considerable stability of the individual calixarene-aromatic amino acids complexes supports their existence also in the case when the amino acids are in a protein. To test this idea the conversion rate, enthalpy and entropy change associated to the structural transition of BSA (Bovine Serum Albumin) were investigated by Differential Scanning Calorimetry (DSC) in the absence and in the presence of calixarene. Results show that presence of calixarene changes significantly both the thermodynamics and the kinetics of the transition of BSA and the information collected for the individual calixarene-amino acid complexes gives insights about the possible processes at molecular level.



Thermodynamic (ΔH and ΔS) and kinetic (E_a) parameters associated to the transition of BSA protein in the presence of 0.05 M calixarene. The different molecular environment was represented by aqueous solutions of ethanol. The ethanol content was varied within the range 0 …0.15 of molar fraction.

Reference

Kunsági-Máté, S.; Lecomte, S.; Ortmann, E.; Kunsági-Máté, É.; Desbat, B. *J. Incl. Phenom. and Macrocycl. Chem.*, (2009) *in press*, DOI: 10.1007/s10847-009-9671-1.

P-24

SELF ASSOCIATION PROCESS OF SOMATOSTATIN CHARACTERIZED BY RAMAN SPECTROSCOPY

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Abstract
Natural Somatostatin-14 (Figure 1) is a small cyclic neuropeptide hormone that is unique in its broad inhibitory effects on endocrine secretions. Natural Somatostatin-14 (as acetate salt) was recently reported to spontaneously self-assemble into stable liquid crystalline amyloid-like nanofibrils under mild and non-denaturing conditions, with proposed implications for its secretion pathway.[1] These non-covalent structures are built on antiparallel beta-sheet hydrogen-bond networks that are developed from the native Somatostatin beta-hairpin.

The Somatostatin aromatic residues were proposed to play a role in the self-assembly process, given their high content in the peptide sequence together with recent works on the structural involvement of aromatic residues in generic amyloid-like fibrils.[2,3] Raman spectroscopy is a pertinent method to follow the conformation and the environment of the aromatic residues during the self-association process of Somatostatin. Here, we followed the self-assembly of natural Somatostatin-14 in aqueous media (15% w/w in water) and over 24 hours. The spectra recorded, respectively, before self-association occurs (t=0) and at the end of the self-association process (t=24h) present main differences on the tryptophan vibrations and the amide I mode. The evolution of the Tryptophan bands shows that during the self-assembly process this residue pass from a hydrophilic environment to a hydrophobic one. In the same times the proportion of beta sheet secondary structure increase. The comparison of the Raman results with other peptide self-assembly modes therefore supports that the Tryptophan variation only observed in the present case could be linked to the reported lateral association of the Somatostatin nanofibrils[1], phenomenon also observed for some other amyloid-like fibrils[4].

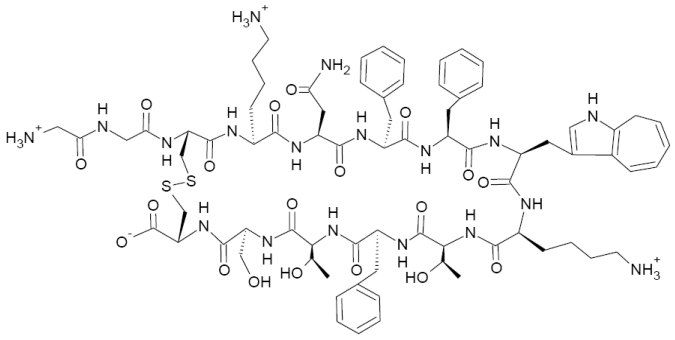

Figure 1 : Structure of Somatostatine

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P-25

PREPARATION AND MODIFICATION OF QUANTUM DOTS AS SELECTIVE PROBES FOR BIOANALYSIS

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Abstract

Quantum dots (QD) show a great potential as photoluminescent probes at cellular level, including in vivo immunolabeling or cellular tracking. Quantum dots are semiconductor inorganic nanocrystals in the size range from 1 to 10 nm. The main advantages of quantum dots, when compared with the conventional organic fluorescent dyes, are practically no photobleaching, wide excitation and narrow emission spectra. Quantum dots have size dependent emission maximum wavelength. Typical materials for the preparation of quantum dots are elements of II – VI or III – V group. We have prepared CdTe water soluble quantum dots of sizes 2 - 4,5 nm by the chemical reaction between $CdCl_2$ and NaHTe at the presence of ligands (e.g. 3-mercaptopropionic acid, 2-mercaptoethylamine). Thus, the photoluminescence emission maxima reach the values 520 - 700 nm. The emission spectra are narrow with the bandwidth of 40-58 nm at the half height of their intensity, while the excitation band lies in the range from 300 to 550 nm. There is a lot of simple conjugation techniques for linking of QD with biomolecules to use them as selective probes in immunolabeling.

We have used zero-length cross-linkers such as 1-ethyl-3-(3-dimethyl-3-aminopropylcarbodiimide (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS) as coupling agents to form peptide bond between carboxylated QDs and an amino group from a protein. As a heterobifunctional cross-linker we have used sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (Sulfo-SMCC) as linking agent to form thioether bond between aminated QDs and thiol group from antibody. The results of the conjugation with functional biomolecules are checked by capillary electrophoresis with LIF detection, will be presented. QDs have a big potential in labeling for the single-cell analyses with LIF detection.

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P-26

ELECTROPHORETIC SAMPLE CLEAN-UP FOR ZONE-ZONE ELECTROPHORESIS SEPARATIONS ON A COLUMN-COUPLING CHIP

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Abstract

Relatively recently, the column-coupling (CC) chip was introduced for the ZE-ZE separation [2]. This particular work was enhancing for potentialities of the ZE-ZE separation, especially, to join a ZE sample clean-up with the ZE separation as reflecting (minimizing) for the analytes. Importantly, our CC-chip technology was based for a hydrodynamically closed separation system [1,2] and, in addition, as operated for the column-switching in the ZE-ZE separations [1].

Practically, the ZE-ZE runs were (a) time-based operations (starting the run from the injection channel) and, in parallel, as linked with (b) the conductivity detection signal (the detection sensor as placed in the separation channel in front of the bifurcation platform [1,2]). The two were switching for the driving current between the relevant driving electrodes (the electrodes as placed for particular separation channels).

Our ZE-ZE experiments were employed for (a) model samples as containing sets of inorganic and organic acids and, in addition, these samples as spiked by (b) biosample matrices (urine). For these ZE-ZE experiments were analytically very benefiting as joining the ZE sample clean-up while followed for a final ZE separation of the chosen analytes.

These operations were very reproducible for both the migration and peak area (height) parameters. For example, the migration times of the analytes (as transferred to the second separation stage) were fluctuated while spanning for 0.2 to 0.5% and their peak areas as characterized for 1.1-4.9% (regarding the RSD values). Very importantly, the recoveries for the analytes were ranged for 94-101% (transfer for the column-to-column runs).

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P-27

CADMIUM REMOVAL FROM WASTEWATERS USING CA-ALGINATE IMMOBILIZED BENTONITE AS ADSORBENT

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Abstract

A commercial bentonite sample from Fort Benton (distributed by Interker-Wein kft., Hungary) was used to remove cadmium ions from synthetic wastewaters. The bentonite sample was used as powder, ($d < 0.2$ mm), without any chemical treatment. We studied the influence of the bentonite quantity, temperature and pH over the process efficiency. The bentonite sample we used proved to be efficient for the removal of cadmium from synthetic wastewaters; removal efficiencies up to 100% were reached.

P-28

MICROCHIP ELECTROPHORESIS FOR FINGERPRINTING ENDOTOXIN CHEMOTYPES FROM WHOLE-CELL LYSATES

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Abstract

Endotoxins (lipopolysaccharides, LPSs) are components of the envelope of Gram-negative bacteria. These molecules, responsible for both, advantageous and harmful biological activity of these microorganisms, are highly immunogenic and directly involved in numerous bacterial diseases in humans such as Gram-negative sepsis. The characterization of endotoxins is of importance, since their physiological and pathophysiological effects depend on their chemical structure. The amphiphilic LPS compounds consist of a hydrophobic lipid region (named Lipid A) covalently linked to the hydrophilic core oligosaccharide with or without the O-polysaccharide region. The differences among the endotoxins from different bacterial serotypes and their mutants include variations mainly within the composition and length of the O-polysaccharide chains.

The proper assignation of the *S* or *R* chemotype of endotoxins is possible by their electrophoretic profiles. The recent microchip electrophoretic methods provide fast characterizations and differentiations of endotoxins directly from whole-cell lysates. The LPS components are visualized either by the interaction with dodecyl sulphate and a fluorescent dye or by a covalently bound fluorescent dye. The labeled endotoxin complexes are analyzed in the Agilent 2100 bioanalyzer microchip electrophoresis system applying the Protein 80 LabChip kit or the High Sensitivity Protein 250 LabChip kit with minor modifications. These chip electrophoretic methods are able to replace the conventional SDS-PAGE with silver staining detection, with the advantage of better sensitivity, high speed and quantification.

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P-29

POLLUTION OF MUSHROOMS AND PLANT SPECIES USED IN SOIL MYCOREMEDIATION

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Abstract

Our paper presents determinations of pollutants (especially heavy metalls) in mushrooms from tailing ponds of Bozanta Mare, near Baia Mare town, in Maramures county. We are also interested in the decreasing rate of the pollutans in soil. The fungi and plants samples were harvest on July-August 2007-2009: *Laccaria laccata, Amanita muscaria, Pisolithus tinctorius, Telephora terrestris, Scleroderma aurantium, Betula verrucosa, Salix caprea, Quercus petraea, Rumex acetosella, Carex sp., Viola tricolor,* etc. We analysed Cu, Fe, Pb, Co, Zn, Cd, Mn by spectrometry with atomic absorbtion used Analyser Perkiner Elmer AA 800. All results were expresed in µg/g dried product at 105^{o}C. All studied samples had high level of these heavy metalls indiferently of plants. These plants and fungi are considered polluting indicators, therefore its may be used for myco/phytoremediation of sublayer on these anthropic areas. This research supported by PN II project: ”Monitoring the action of the soil’s microbiot for considering its use in the ecological rehabilitation of the decantation ponds”.

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P-30

(ULTRA)TRACE DETERMINATION OF BROMIDE IN DRINKING WATER BY CAPILLARY ISOTACHOPHORESIS- ZONE ELECTROPHORESIS

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Abstract
Bromide present in drinking waters is linked with bromate as formed on ozonation. This disinfection by-product is of a general healthcare concern. Due to this, the EU countries and the US EPA regulate the concentration of bromate in drinking water via maximum contaminant levels (10 μg/l) and taken as an enforceable standard. In addition, the Food Codex regulates bromate at a 3 μg/l concentration in bottled mineral and source waters while treating the bottled waters by air and ozonation. It is very logical to develop an adequate analytical tool for an (ultra)trace determination of bromide.
Our study was reflecting to develop an electrophoretic procedure for this analyte and still reachable for an (ultra)trace concentration level. In this context, we were favoring to join capillary zone electrophoresis (CZE) with isotachophoresis (ITP) sample pre-treatment. This ITP-CZE combination was employed for the photometric detection at a 200 nm wavelength in the CZE stage.
The ITP-CZE separation was favored a relatively low pH (2.8) for the CZE stage, especially, to achieve a highly selective separation system. In addition, the ITP stage, used the leading electrolyte at pH=3.7, was applied very selectively by using an zwitterionic detergent [3-(N,N-dimethyldodecylammonio)propane sulfonate, DDAPS]. In fact, this ITP selector was employed to influence the effective mobility of bromide as resolvable for major anionic constituents as typically found in drinking waters (e.g., chloride, sulfate and nitrate). In parallel, the ITP stage was performed for a very effective sample clean-up (the matrix constituents were removed outside from the separation system while transported electrophoretically).
Very good repeatabilities for the migration times of bromide (0.8% RSD) as well as the peak areas (0.4 % RSD) were typical for the (ultra)trace analysis as based on ITP-CZE. Our limit of detection for bromide was estimated at a 300 ng/l concentration for the CZE stage while this analyte was still accompanied by chloride at a 150 mg/l concentration (loading a 30 μl volume of the sample). A series of table and tap water samples were analyzed to assess a practical applicability of the ITP-CZE method as clearly documented for the (ultra)trace quantitation. Recoveries of bromide in the analyzed drinking waters were ranged in the interval 96-108%.

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P-31

TRACEABILITY OF HEAVY METALS ALONG OF SOIL-PLANT-HONEY CHAIN

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Abstract

The paper presents the influence of the soil's pollution with heavy metals influences the mineral content of honey bees and implicitly the honey.

The study, developed during a month, was performed in situ by location the bee hive in two areas: non-polluted area (as reference area) and a strongly polluted area with heavy metals.

Samples of soil, mellifera plants (*Red and white clover*, *Common Yarrow*, *European field pansy*, *White Deadnettle*), bees and honey were collected from both areas and analyzed using atomic absorption spectrometry method in order to establish the content of heavy metals (Pb, Cu, Zn, Cd, Mn, Ni, Cr).

The concentration of heavy metals in soil coming from reference area is reflected in low level of metallic content in studied plants, bees and honey they produced.

Analysis of samples coming from polluted area indicate high concentration of Fe (12366.94 mg/Kg), Cu (301.42) and Zn (60,19 mg/Kg) in soil. As consequence, high concentrations of those metals were founded in meliffera plants, bees and honey. The presence in honey, bees and plants of low level of some metals (Pb, Mn) can be correlated with presence in soil of theirs insoluble chemical combinations.

Translocation of hive from polluted area to un-polluted area contributed to a decrease of mineral content in bees and honey.

P-32

ADSORPTION OF DIFFERENT METAL IONS BY A NATURAL ZEOLITIC TUFF

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Abstract

Natural zeolites, known for their excellent sorption properties towards metal cations, are widely used for the purification of wastewaters. The isotherms of adsorption for different metal ions (lead, copper, zinc, potassium, manganese) from aqueous solutions by natural zeolitic tuff has been investigated. Batch experiments at constant temperature and hydrodynamic conditions have been performed. The maximum adsorption capacities were determinated. A decrease of the initial metal ions concentration in aqueous solutions induce the decrease of the adsorption capacities. Using the distribution coefficient (calculated as the ratio of the metal ion concentration in the solid phase to the concentration in the liquid phase at equilibrium), the strength of metal bonds with the zeolite were evaluated.

P-33

GATHERING PHYSICOCHEMICAL DATA FOR DESIGNING BIOFUEL PROPELLED GRAIN DRYERS

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Abstract

The freshly harvested agricultural crops like corn, sunflower seed, and rape often need drying to avoid deterioration during storage. The large scale drying is a highly energy consuming, therefore expensive process. There are different kinds of crop dryers on the marker mostly using natural gas as energy source. A research work aiming to develop a new kind of crop drying equipment of very much cost efficient operation is in progress. The dryer to be developed uses hybrid fuel propelled burner. It operates partly on agricultural waste biomass fuel partly on natural gas.

In our laboratory measurements are carried out in interaction with the machinery developers to provide the back ground data and support needed for designing the different units of the apparatus.

Part of this studies deals with the drying process of different grain species. In this work thermo gravimetric measurements have been employed using temperature scanning with different scanning rates as well as recording grain mass - time dependences at constant temperature. The data obtained in these experiments were used to estimate the effective diffusion coefficient of water inside the different kinds of cereal. The activation energy of diffusion of water could be also guessed using a rough diffusion model.

Our presentation will describe the measuring methods and sample pretreatment procedure used. It will be shown some of the characteristic dependences obtained and will introduce the rough model employed for obtaining the physicochemical values necessary for characterize the drying process of the individual grain particles.

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P-34

EFFECTS OF THE SANDWICH INJECTION PARAMETERS ON THE pH GRADIENT IN CIEF ANALYSIS WITH MS DETECTION

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Abstract
Isoelectric focusing within a fused silica capillary (cIEF) is a well-known, high-resolution separation technique for the analysis of amphoteric compounds, such as peptides or proteins. The analytes are separated according to their isoelectric point (p*I*), in a pH gradient established by ampholytes under the influence of an electric field. cIEF can be performed in uncoated capillary, where the focusing step and the mobilization step are carried out simultaneously and the pH gradient moves towards the cathode with the support of the electroosmotic flow (EOF). In most cases the analytes and the carrier ampolytes are introduced in mixtures. cIEF has been used extensively with conventional UV detection; however, the use of cIEF in conjunction with mass spectrometry (cIEF-MS) provides a more effective identification and structural characterization of the analytes.
We have previously worked out a cIEF method applying sandwich injection set-up, where the analysed sample compounds were placed between two ampholyte zones before the analysis. This injection protocol can be used for the effective separation of amphoteric compounds having p*I* values outside the pH range of the applied ampholyte. Our aim was to connect this "sandwich" cIEF method with mass spectrometric (MS) detection and to examine the influence of several parameters on the pH gradient.
For the tracing of the pH gradients the low-molecular-mass p*I* markers (substituted aminomethylphenol dyes with p*I*s 5.3, 6.4, 6.6, 7.9 and 10.4 respectively) were used. Separations were performed using ampholytes with narrow and broad pH ranges in uncoated capillary. During the separations, the evolving pH gradient was affected by the length of ampholyte zones and the type of the ampolyte solutions.
In order to combine the cIEF method with MS detection volatile solution were applied as anolyte and catholyte (formic acid and ammonium hydroxide), since volatile solutions are preferred in MS analysis. Although, the profiles of the isoelectropherograms with different detections (UV and MS detection) were similar, broader peaks were obtained within shorter separation time with the MS detection. The broadening of the peaks is due to the substantial dilution of the peak zones by the sheath liquid.

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P-35

SEPARATION OF HUMIC ACIDS BY COUPLING CAPILLARY ISOTACHOPHORESIS WITH ZONE ELECTROPHORESIS UNDER MICELLAR CONDITION

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Abstract
Capillary isotachophoresis-zone electrophoresis (ITP-CZE), using a column-coupling technique with a hydrodynamically closed separation system, was studied for the separation of humic acids (HAs). This electrophoretic technique was performed in an anionic separation mode with UV photometric detection (254 nm), especially, in the CZE stage.
In dependence for a range of pH values and the ionic strength of HAs, as present in aqueous solutions, tend to aggregate. However, the separations at a high pH were indicated to eliminate this aggregation. In fact, acidic groups of HAs are ionized completely (carboxylic) and to high degrees (phenolic) at pH=10. This pH value is attributable to eliminate efficiently for the aggregations of these constituents.
HAs constituents were separated isotachophoretically into 5 fractions. On the other hand, a resolution of HAs in the CZE stage (combining ITP-CZE) indicated only a low impact of pH on the effective mobilities of HAs. In general, the electrophoretic profiles of HAs are reflected for typical one very broad peak (humic ‘‘hump’’) in the CZE stage.
In the present work, we studied for the BGE solutions as containing zwitterionic detergent (dodecyldimethylammoniopropanesulfonate, DDAPS). Forming DDAPS micelles was very significantly enhanced for the peak resolution in the CZE stage. The 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 water. However, in an electrolyte, an imbalance between the anion- and cation- partition, induces surface charges and surface potential. The ITP-CZE separation of humic substances as using the BGE while contained DDAPS at a lower concentration [critical micellar concentration (CMC) = 2-4 mmol/l] did not influence the CZE profile (very likely no impact on the effective mobilities of HAs). Considerable changes in the CZE electropherogram profile, in the presence of higher concentration of DDAPS in the BGE, were observed. This important fact indicates that interaction between DDAPS micelles and HAs can be change significantly for the effective mobilities of humic and humic-like substances.
Hydroxyethylcellulose (HEC) was used in the electrolyte solutions to suppress for the electroosmotic flow at a low concentration (e.g., at a 0.1%). It should noted as HEC, while present in the electrolyte solution at a very high concentration, has no specific impact on the effective mobilities of HAs in the CZE stage of the ITP-CZE separation.

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P-36

ELECTROCATALYTIC OXIDATION OF DOPAMINE AND SEROTONINE AT CARBON NANOTUBE PASTE ELECTRODE MODIFIED WITH FEROPHTHALOCYANINE

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Abstract

Dopamine (DA) and Serotonine (5-HT) are among the most important catecholamines. They belong to the family of excitatory chemical neurotransmitters that play very important roles in the function of the central nervous, renal, hormonal and cardiovascular system. Low levels of DA have been found in caudate of patients with Parkinson's disease. Unusual levels of monoamines have been reported in patients suffering from other diseases such as schizophrenia, HIV infection [1] etc. Therefore, it is of great clinical importance to measure DA level in the extracelluar fluid in order to monitor neurotransmission process and diagnose Parkinson's disease. Various methods have been applied to detect DA, such as spectrophotometry [2], HPLC [3], ion chromatography [4] and so on. Electrochemical methods have been considered as one of the most potential approaches to this purpose because of their high sensitivity, simplicity and good electrochemical property of DA. The irreversibility of its electrochemical property results in a large overpotential for oxidation at the conventional electrode. In order to lower the potential range needed for determination, working electrodes modified with electrocatalytic layers have been employed. Among them catalytic layers formed by electric pretreatments [5], or by addition of mediator species like Prussian Blue, pretreated nano-materials [6], different polymers [7] etc. are the most often selected ones. In order to increase selectivity perm selective membranes with ion exchange character like Nafion [8] were applied in DA determinations. Metallophthalocyanine (MPc) and its derivatives are well known as electrocatalysts for many reactions [9]. However, the use of MPc complexes as electrocatalysts for the determination of monoamine neurotransmitters has not received much attention yet. In our recent work the electrocatalytic action of iron phthalocyanine ($Fe^{II}PhC$), in voltammertic oxidation of DA and serotonin (5-HT) has been investigated. In these studies Carbon Nanotube Paste Electrodes (CNTPE) modified with Ferophthalocyanine have been employed using different voltammetric techniques. The interferences of other electroactive species like ascorbic acid (AA), paracetamol etc. appearing in biologic samples have been also investigated.

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P-37

ANALYSIS AND CHARACTERIZATION OF SELECTED HUMIC ACIDS USING RP-HPLC METHOD

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Abstract

Humic substances (HS) are complex mixture of amorphous, yellow to black colored, hydrophilic, polyelectrolyte-like, polydisperse bio-macromolecules and probably no their two molecules are identical. Characteristic feature of these analytes is created by their diffuse non-distinct analytical signal. LC method designed around RP-HPLC using stepwise gradients of dimethylformamide (DMF) in buffered aqueous mobile phase and a wide-pore (30nm) octadecylsilica column [1,2] had been applied to the analysis of 3 different samples of HSs (one type of humic acid isolated from soil [3,4] and two types of commercially available HSs). Tandem combination of spectrophotometric (DAD) and fluorimetric detection was used to get more detailed information on chromatographic behavior of HSs.

The results showed that ten-step gradient can induce distinct features of HSs .

Combination of very good DMF solvating and disaggregating properties for HSs together with wide pore RP sorbent improves surface interactions of the analytes and suppresses influence of size exclusion effects. Thus it provides reproducibility of characterization profiles and robustness of the methods.

Individual fractions obtained by the using RP-HPLC method were analysed by the same chromatographic method, which means re-injection of all 11 collected fractions from every three analysed HS samples. Obtained data indicate, that this (mode) application of using RP-HPLC system could be employed as a separation system for get more detailed characterization of such complicated natural biopolymers such as analysed HS and obtain so more information about their attributes.

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P-38

FOCUSING OF METALS IN LIGAND FIELD STEP GRADIENT

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Abstract
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 ions of alkali earth metals /Mg, Ca, Sr, Ba/ were pre-concentrated from the mixture and analyzed. Focusing of metals was carried out in ligand field step gradient, which was realized by an addition of a convenient ligand agent to the regular stationary pH step gradient [1].
During the first step, the metal ions were continuously dosed into the column, where they were selectively trapped on the stationary ligand field step gradient in the form of non moving zones of chelate 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 e.g. by ITP method with conductivity or photometric detection.
The proper electrolyte system for the dosing /mode IEF/, mobilizing /mode MBE/and analytical step /mode ITP/ were developed and realized.
The selectivity of the trapping can be regulated by a choice of the pH and convenient complexing agents. As a sample analytes served model real mixtures of alkali earth metals. The proposed method enable increase of detection limit is 5-29x in comparison to classical methods – e.g. ITP.

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ON-LINE COMBINATION OF CAPILLARY ZONE ELECTROPHORESIS WITH MASS SPECTROMETRY USED TO ANALYSIS OF BUSERELIN IN HUMAN URINE

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Abstract

For majorities of biological samples are reflecting for advanced analysis tasks. Of these are attributed for samples as included for several hundreds of constituents and, in addition, representing different chemical/biological properties and still covering for very significant concentrations of the analytes. Drug analytes and/or their metabolites, are usually present in urine or serum and provided for trace concentration levels. These two categories of the analytes indicate a combination of powerful separation techniques with a sensitive and/or selective detection technique. CZE-MS is proved to be a powerful analytical technique, for example, as separating and identifying biologically important compounds such as proteins and peptides as joined with analytical constituents of different complex biological mixtures. Main advantages of this coupled system are in high separation efficiencies, short analysis times, high detection selectivities and sensitivities and still consuming low sample amounts and almost negligible consumptions of the electrolytes. Busereline (5-oxoPro–His–Trp–Ser–Tyr–D–Ser(t-Bu)–Leu–Arg–Pro–NHC2H5), a synthetic analog of natural gonadotropin-releasing hormone, is used to treat prostate or breast cancer [1, 2, 3]. Buserelin is excreted in urine and bile as the unchanged drug (66 % of the dose) and its metabolites (28 % of the dose in 24 hours). A small part of the dose (17 to 32 %) is detected for urine and administrated Busereline by intravenous or subcutaneous doses. Dose, for example in prostatic carcinoma, is 500 μg (of the base) applied subcutaneously every 8 hour for 7 days, then 200 μg into each nostril every 8 hour [2].

This work is dealing with the analysis and quantification possibilities of buserelin present in complex biological matrix (urine) by using CZE-ESI-MS with sheath-flow interface.

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P-40

SELECTION OF PHAGE-DISPLAYED RECOMBINANT SCFV ANTIBODIES SPECIFIC FOR SPLA2 FROM *VIPERA AMMODYTES MERIDIONALIS*

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Abstract

Snake venoms are one of the most complex mixtures of proteins exhibiting a variety of pharmacological effects such as neurotoxicity, myotoxicity, cardiotoxicity, platelet aggregation, hemolysis, anticoagulation and others [1]. The most toxic among them are secreted phospholipase A_2 ($sPLA_2$) enzymes (phosphatide *sn-2* acylhydrolase, PLA_2, EC 3.1.1.4). $sPLA_2$ superfamily of enzymes catalyze specifically the hydrolysis of the 2-acylester bond of 1,2-diacyl-3-*sn*-phosohoglycerides in a calcium-dependent manner, releasing fatty acids and lisophospholipids [2].

$sPLA_2$ enzyme is the main and most toxic component of the neurotoxin vipoxin isolated from the venom of *Vipera amm. meriodionalis*. The neurotoxin is a heterodimeric postsynaptic ionic complex composed of two protein subunits – a basic and strongly toxic His48 $sPLA_2$ enzyme and an acidic, enzymatically inactive and nontoxic component. Both subunits bind one calcium ion according to the spectroscopic studies and display a high degree of sequence homology [3].

Neutralization of snake venoms and toxins has been investigated for many years using different approaches including antibodies, non-immunologic inhibitors, and natural products derived from plants and animals, as well as synthetic drugs.

Here we demonstrated the use of large nonimmune human scFv libraries named Tomlinson I + J (Cambridge, UK) for the selection of recombinant specific antibodies able to inhibit $sPLA_2$ from vipoxin. Numerous clones were identified as capable of inhibiting this activity *in vitro* and will be used to determine the epitopes associated with biological activity, to distinguish the biological effects and enzyme activity of $sPLA_2$, and to enlighten the role of catalytic activity for the toxicity of venom $sPLA_2$.

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P-41

CAPILLARY ELECTROPHORESIS WITH CONTACTLESS CONDUCTIVITY DETECTION AS PROMISING TOOL FOR DETECTION OF INBORN ERRORS OF ORGANIC ACIDS METABOLISM

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Abstract

Low-molecular organic acids play an important role in many biochemical and physiological processes in the body. Organic acids are intermediate metabolites of all organic cellular components as amino acids, lipids, carbohydrates, nucleic acids and steroids. The organic acidurias are a biochemically heterogenous group of inborn errors of metabolism [1]. They are characterized by the accumulation of abnormal amounts of organic acids and its metabolites in urine. The pathologic state is related to the loss of specific gene function. Gas chromatography/mass spectrometry has been routinely used as a screening method for diagnostic of metabolomic disorders [2]. This methodology is complicated, requires expensive laboratory equipment and is also time consuming.

Capillary electrophoresis (CE) with contactless conductivity detection (CCD) should be a promising alternative for quick monitoring of the metabolomic disorders [3]. The main advantages of electrophoretic separation are low sample consumption, minimal pretreatment of biological material before analysis, high separation efficiency and high speed of analysis. Moreover, the recent progress in the development of CCD for CE permits the sensitive detection of all organic acid without necessity its derivatization [4]. The CE/CCD determination of cca 25 organic acid in untreated urine is demonstrated in this contribution. The limits of detection of this methodology range on the micromolar level.

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P-42

GAS CHROMATOGRAPHY-MASS SPECTROMETRY DETERMINATION OF TESTOSTERONE AND ITS METABOLITE IN URINE

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Abstract
Testosterone is used clinically for androgen replacement therapy, however for its anabolic effect is widely abused in sport to enhance muscle development, strength, or endurance, physical apperarence [1].
The measurement of testosterone is used to evaluate endocrinne activity in men and to control of anabolic steroids abuse to prevent of side effect. Epitestosterone - the metabolite of testosterone has attracted the attention as a reference substance in the control of testosterone abuse. The ration of urinary testosterone to epitestosterone in adults became a marker of detection of exogenously administered testosterone and abuse of other anabolic steroids (androstendione and dehydroepiandrosterone [2].
A gas-chromatography and mass spectrometry method for quantification testosterone and epitestosterone in urine is described. For isolation of free and conjugated form of this steroids, the liquid-liquid extraction, deconjugantion and derivatization methods were developed. The recovery in urine was 90 - 95 %. The deconjugatin step was realised by enzymatic hydrolysis with β-glucuronidase. Isolated steroids were derivatised by MSTFA and analysed on ULTRA 1 column (m/z 432, 446). The limit of quantification was 0,5 ng/ml. This method was applied to clinical samples for determination of physiological ratio of testosterone and epitestosterone.

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P-43

DETERMINATION OF DIFFUSION COEFFICIENTS OF GLUCOSE IN SOLUTIONS AND GELS BY ELECTROCHEMICAL TOF METHOD

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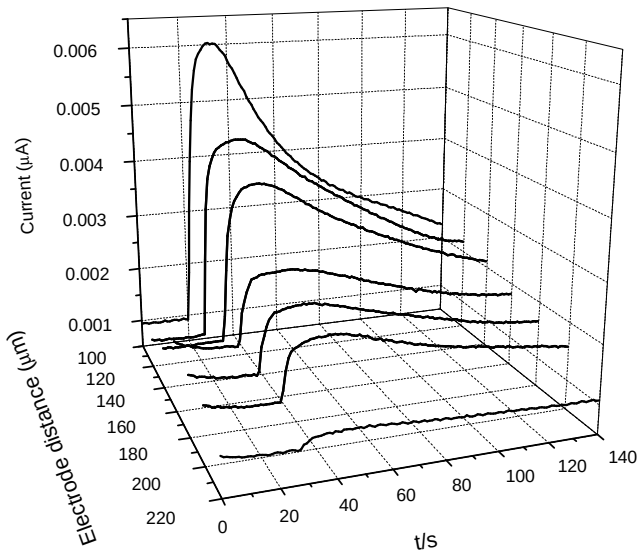
Abstract

The diffusion coefficient of glucose in different media is an important parameter in life sciences, as well as in biotechnology and microbiology. In this work a simple, fast method is proposed that is based on the electrochemical time of flight (EC-TOF) principle. In most of the earlier performed time of flight experiments constant flight distance was applied. In the present work Scanning Electrochemical Microscope (SECM) has been applied as measuring tool. Using the SECM the flying distance could be changed with high precision. Making measurements with several flight distances more accurate and reliable values could be obtained for solutions as well as for gels. The conventional voltammetric methods are not applicable for glucose detection. In our work electro catalytic copper oxide coated copper microelectrodes and micro sized amperometric enzyme sensors were used as detectors, while micro droplet ejecting pneumatically driven micropipettes were used as source.



Current-time recordings obtained in case of different *d* (flight) values using glucose enzyme electrode for detection in amperometric mode. (Electrode potential 0.65V vs. silver quasi reference).

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P-44

PROTEASE AND CELLULASE ACTIVITY IN THE BOZANTA MARE TAILING POND FROM MARAMURES COUNTY

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Abstract

We studied the protease and celulase activity in the tailing pond Bozanta Mare, situated in the N-W area of the Maramures county. This tailing pond was the result of the intense mining activity from this area. The activity of those two enzymes was found to depend on the collecting area from the tailing pond and also on the depth on which the soil samples were collected. Moreover, the protease and celulase activity was found to be deeply influenced of the vegetation. The tree vegetation, especialy, intensify the protease activity from the inferior layers of the tailing pond.

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OPTICAL pH SENSOR BASED ON A DUAL LIFE TIME REFERENCING (DLR) METHOD

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Abstract

The development of optical pH sensors has gain a considerable attention because of their potential use in many different fields. While an electrochemical pH determination is acknowledged and well established method, there are some applications where their use is not advantageous, because they are interfered by electromagnetic field, radioactive radiation, or they have a short life time in continuous pH monitoring. In these cases the optical pH determination gives an alternative solution.

Optical parameters that can be exploited are: absorbance, reflectance and fluorescence. In the case of the fluorescence the most commonly used parameter is fluorescence intensity, nevertheless it also has some drawbacks. It requires additional calibration, it is influenced by dye leaching, photo bleaching, the sample turbidity can abrogate the measured values. Several methods have been developed to over come these problems like: intensity ratio measurements, or the latest Dual Lifetime Referencing (DLR) method[1]. It is a new principle to reference fluorescence intensities via fluorescence decay times. The DLR method uses two fluorescent dye with overlapping spectroscopic properties, one pH-sensitive, short-lived indicator and a pH-insensitive reference dye with a decay time in the μs or ms range.

In the present work we have synthesized and use 1, 8-Naphthalimide derivative as a pH sensitive dye[2], and Rhutenium complex as reference fluorophore [3]. Two dyes has been co immobilized in the sol-gel matrix and has been tested for spectral characteristics, repeatability, reversibility, response time, selectivity and the effect of ionic strength.

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P-46

EFFECT OF SOIL REACTION ON SELENIUM CONTENT IN WHEAT IN NITRA REGION

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Abstract

In our work the Se content was evaluated in the soils of Nitra region and in the grains of wheat grown on these soils as well. Samples were collected during harvesting from 28 localities. The Se content was determined by AAS method using the electrothermic atomization vapor both in the grains and in the soils. The average Se content in the grains of wheat of Nitra region was 53µg.kg^{-1}. This value exceeds the average content in Slovakia (28,9 µg.kg^{-1}). The Se distribution in the whole area of Nitra region was different. In Nitra district in the soils with alkaline soil reaction (chernozem, fluvi-gleyic Pheozem) the Se content was high. The very low Se content in grains was determined in the districts of Topoľčany and Zlaté Moravce, although its soil content was high (brown soil with medium acid or neutral soil reaction). The average value of total soil Se content of Nitra region exceeded the limit value 1,25 – times. The Se grain content grown on the soil of the north of Nitra region was non-detected despite the fact that its soil content was over-limited. The Se intake by the plants depends on its form in the soil and on the soil type as well.

Notes