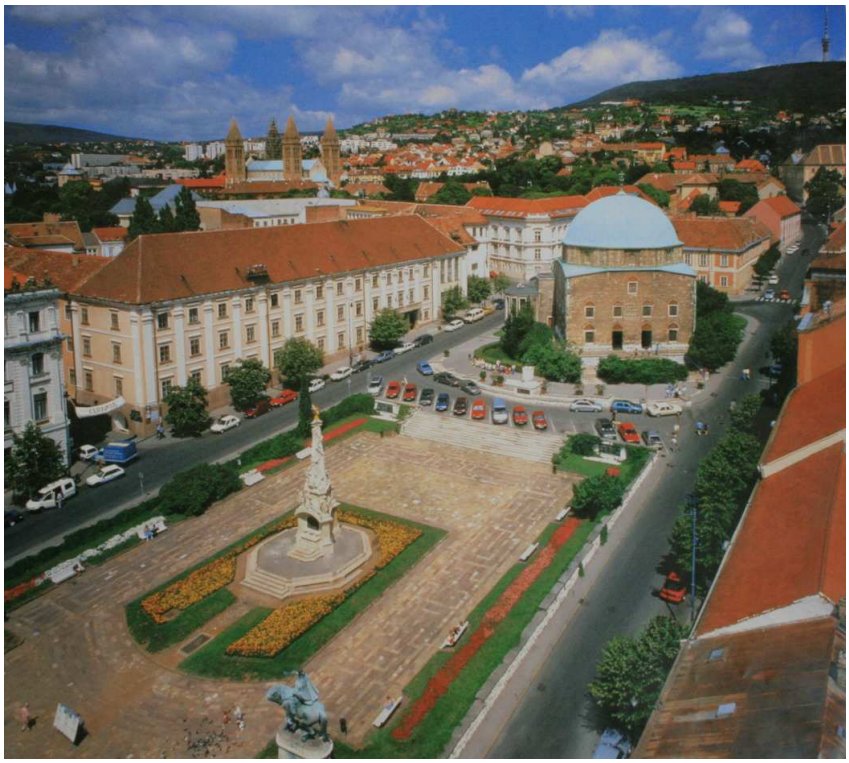
CECE 2010

7TH INTERNATIONAL INTERDISCIPLINARY MEETING ON BIOANALYSIS



PROGRAM AND ABSTRACTS

October 14 – October 17, 2010

PÉCS

HUNGARY

CECE 2010

7TH INTERNATIONAL INTERDISCIPLINARY MEETING ON BIOANALYSIS

Organized by

University of Pécs
and
Regional Center of the Hungarian Academy of Sciences

PROGRAM AND ABSTRACTS

October 14 – October 17, 2010
PÉCS

HUNGARY

Edited by

Ferenc Kilár and Ágnes Dörnyei

ISBN 978-963-642-341-3

Printed by Kontraszt Plusz Kft., H-7621 Pécs, Jókai u. 11., Hungary.

Welcome to this year's CECE

This symposium is the seventh 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 opportunity for discussions and presentations of new results in bioanalytical science.

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://cece2010.pte.hu

Organizing Committee

Ferenc Kilár – Chairman
František Foret – Co-Chairman
Attila Felinger
Balázs Csóka
Tímea Fekete
Levente Kilár
Anita Bufa
Ágnes Dörnyei
Ibolya Kiss

Registration fee

The registration fee is 15000 HUF (17000 HUF after September 1), 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 reception on Thursday evening and dinner on Friday are organized in the Hotel Hunyor (Jurisics Miklós utca 16.) for the registered participants. The coffee breaks and lunches on Friday and Saturday are organized in the symposium-site (Vasváry Villa, Jurisics Miklós utca 44.) for the registered participants.

The Organ concert will take place in the Cathedral. Registration should be made at the Registration desk.

The Symposium dinner will take place in the Somogyi Pince (Hunyadi János utca 12. 7625 Pécs, http://www.somogyipince.hu/pecsi-latvanypince)

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 from Thursday to Sunday (14-17 of October, 2010) is covered by the Symposium for all registered participants (whose registration has been acknowledged by the organizers). Other request (longer stay) should be covered by the participant.

Venue

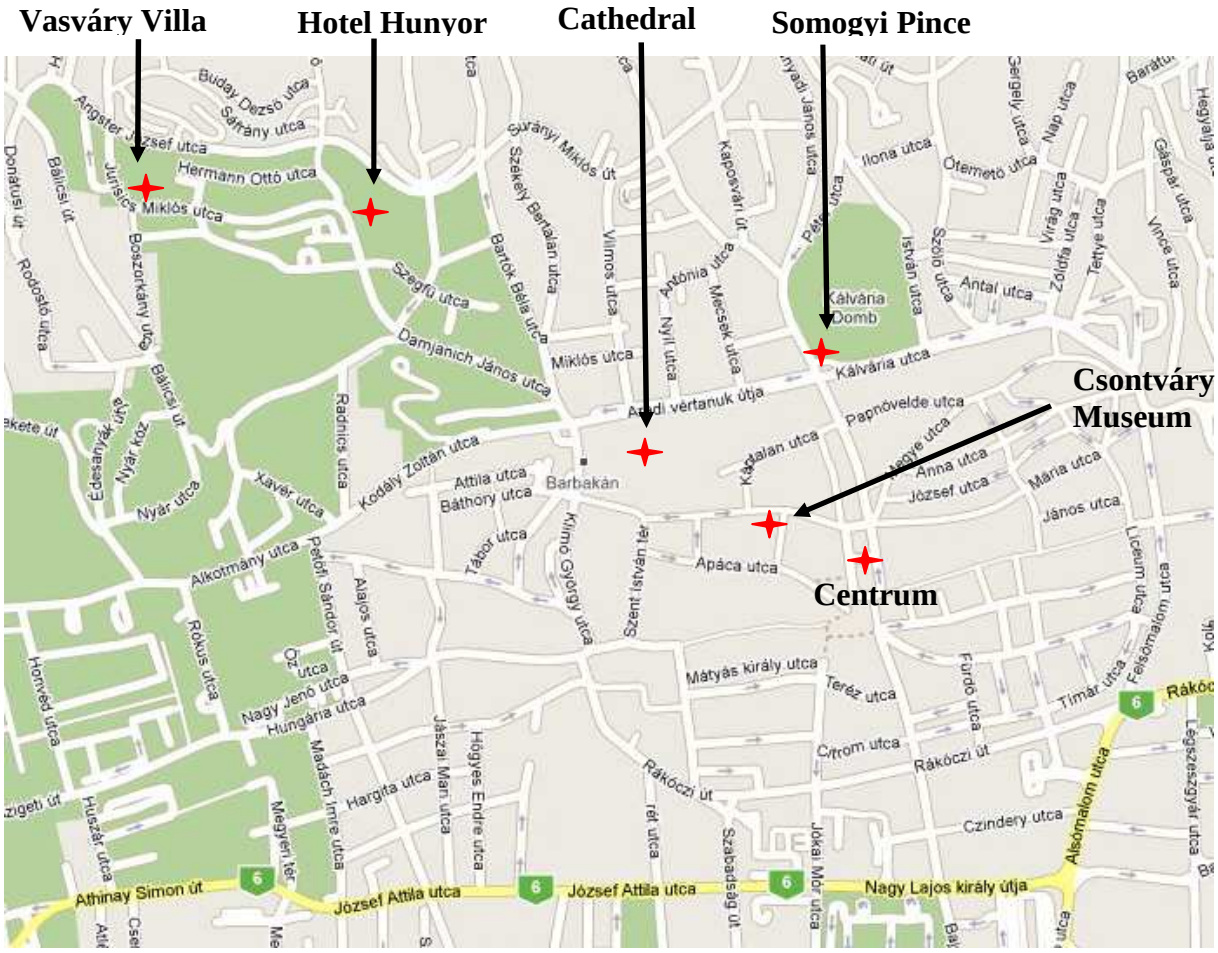
The symposium will be held at

VASVÁRY VILLA

(Regional Office of the Hungarian Academy of Science)

H-7624 Pécs, Hungary

Jurisics Miklós utca 44**.**



http://cece2010.pte.hu

Opening hours of the Registration desk

October 14, 2010: 15:00-19:00 (Hotel Hunyor, Jurisics Miklós utca 16.)

October 15, 2010: 8:00-10:00(Vasváry Villa, Jurisics Miklós utca 44.)

Language: English

Sponsors

PÉCSI TUDOMÁNYEGYETEM

EURÓPA KULTURÁLIS FŐVÁROSA 2010 – PÉCS

Program

October 14, 2010

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

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

October 15, 2010

8:00 – 10:00 Registration
Vasváry Villa, Pécs, Jurisics Miklós utca 44.

08:30 Opening of the Symposium
Vasváry Villa, Conference Hall

Chairman: Ferenc Kilár

08:45 L-01 *František Foret*
Academy of Sciences CR, Brno, Czech Republic
Miniaturization in bioanalysis

09:20 L-02 *Bezhan Chankvetadze*
Tbilisi State University, Tbilisi, Georgia
Mechanistic aspects of enantioseparations of selected chiral drugs in aqueous and non-aqueous capillary electrophoresis

09:55 L-03 *Pier Giorgio Righetti* and Egisto Boschetti*
Politecnico di Milano, Milano, Italy
The proteome buccaneers: how to unearth your treasure chest via combinatorial peptide ligand libraries

10:30 Coffee break

Chairman: František Foret

11:00 L-04 *Stefan Mittermayr, Jonathan Bones, Zoltán Szabó, Pauline M. Rudd, Barry L. Karger and András Guttman**
Northeastern University, Boston, USA
Capillary electrophoresis based analytical and structural prediction tools for glycan analysis

11:35 L-05 *Johan Roeraade*
KTH - Royal Institute of Technology, Stockholm, Sweden
Miniaturized technologies in biochemical analysis

12:10 L-06 *Attila Gáspár*, Andrea Nagy and István Lázár*
University of Debrecen, Debrecen, Hungary
Integration of ground aerogel particles as chromatographic stationary phase into microchip

12:45 Lunch

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

Chairman: Bezhan Chankvetadze

15:00 L-07 *Stellan Hjertén*
Uppsala University, Uppsala, Sweden
The requirements for high resolution and high reproducibility in capillary electrophoresis

15:35 L-08 *Gyula Vigh* and Edward Tutu*
Texas A&M University, College Station, USA
Heptakis-(2-O-sulfo-3-O-methyl-6-O-acetyl)-cyclomaltoheptaose, a single-isomer chiral resolving agent for enantiomer separations by capillary electrophoresis

16:10 L-09 *Wolfgang Thormann*
University of Bern, Bern, Switzerland
Determination of alcohol markers in body fluids by CE

16:45 L-10 *Karel Kleparnik*
Academy of Sciences CR, Brno, Czech Republic
Advances in bioanalytical application of nanotechnologies

17:30 Dinner, Hotel Hunyor, Restaurant

19:00 Organ concert, Cathedral (Dóm tér) (short tour in the Cathedral at 18:40)

October 16, 2010

Chairman: Karel Kleparnik

08:30 L-11 *Marek Trojanowicz*
University of Warsaw, Warsaw, Poland
New trends in design of chiral sensors and biosensors

09:05 L-12 *Ziad El Rassi* and Subhashini Selvaraju*
Oklahoma State University, Stillwater, USA
Liquid phase separation strategies for in-depth proteomics profiling

09:40 L-13 *Stefan Paireder, Josef Bailer, Wolfgang Werther, Erich Schmid, Beatrix Patzak and Margit Cichna-Markl**
University of Vienna, Vienna, Austria
Comparison of different procedures to extract DNA from long-term preserved formalin fixed tissues for PCR amplification

10:15 Coffee break

Chairman: Ziad El Rassi

10:45 L-14 *István Földi, Géza Müller, Gábor Juhász and Tamás Janáky**
University of Szeged, Szeged, Hungary
Selection of experimental animals for proteomic studies

11:20 L-15 *Yingli Liu, Yasmina Mekmouche, Eloine Npetgat, Viviane Robert, Pierre Rousselot Pailley, Sana Ullah and Thierry Tron**
Université Paul Cézanne, Marseille, France
Engineered laccase: artificial enzymes with new properties

11:55 L-16 *Éva Szökő*, Tamás Tábi and Zsolt Wagner*
Semmelweis University, Budapest, Hungary
Difficulties of CE-LIF in the analysis of biological samples

12:30 Lunch

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

Chairman: Attila Gáspár

14:30 L-17 *Staffan Nilsson*
Lund University, Lund, Sweden
Development in "Airborne Chemistry" & Protein Epitope MIP PSP-CEC

15:05 L-18 *Attila Felinger*, Ibolya Kiss and Péter Vajda*
University of Pécs, Pécs, Hungary
Performance of modern HPLC stationary phases in bioanalysis

16:00 Guided Tour in Cella Septichora / Csontváry museum / Munkácsy Trilogy

19:00 Symposium Dinner with wine tasting, Somogyi Pince (H-7621 Pécs, Hunyadi út 12.)

October 17, 2010

Departure

LIST OF POSTERS

P-01 *Melinda Andrási*, Rose Bustos, Attila Gáspár, Frank A. Gomez, Álmos Klekner*
University of Debrecen, Debrecen, Hungary
Analysis and stability study of temozolomide using capillary electrophoresis

P-02 *Katalin Balogh*, Péter Ács, Barna Kovács*
University of Pécs, Pécs, Hungary
Determination of chloride ion by using fluorescent nanobeads

P-03 *Ágnes Blaskó*, József Belágyi, József Deli, Csaba Vágvölgyi and Miklós Pesti*
University of Pécs, Pécs, Hungary
EPR studies in yeast cells: effect of carotenoids on plasma membrane dynamics

P-04 *Péter Buzási*, Dávid Szabó, Ferenc Kilár and Béla Kocsis*
University of Pécs, Pécs, Hungary
Video-microscopy Capillary Electrophoresis System

P-05 *G. Ciurescu, J. Izquierdo*, J.J. Santana*, S. González, D. Mareci, D. Sutiman, R.M. Souto**
University of La Laguna, La Laguna (Tenerife), Spain
Use of Scanning Electrochemical Microscopy (SECM) for the local analysis of surface reactivity in biomedical Ti20Mo alloy exposed to different electrolytic environments

P-06 *Zsuzsanna Czibulya*, Lívia Nagy, Ágnes Németh, Géza Nagy*
University of Pécs, Pécs, Hungary
The development of inverse voltammetric method for estimation for heavy metal content in tortuose environmental media

P-07 *N. El Bakkali-Tahéri*, L. Brisson, V. Robert, M. Réglier, E.-H. Ajandouz and A. J. Simaan*
Université Paul Cézanne, Marseille, France
ACC Oxidase: characterization and inactivation mechanism

P-08 *Viktor Farkas*, Krisztina Deák, Alžbeta Hegedűsova, Tímea Pernyeszi*
University of Pécs, Pécs, Hungary
Biosorption of lead(II) ions by pretreated biomass of *Phanerochaete chrysosporium*

P-09 *Krisztina Honfi* ,Rita Szabó, Valéria Hodován, Imre Dékány, Tímea Pernyeszi*
University of Pécs, Pécs, Hungary
Phenol and lead adsorption on bentonite and modified bentonite by benzyldodecyldimethylammonium bromide in aqueous solution.

P-10 *Javier Izquierdo*, Livia Nagy, Géza Nagy, Ricardo M. Souto*
University of La Laguna, La Laguna (Tenerife), Spain
Detection of electrochemically active species being consumed and produced in metals by scanning electrochemical microscopy

P-11 *Silvia Jakabová* and Attila Felinger*
University of Pécs, Pécs, Hungary
Stationary phase characterization with total pore blocking and inverse size exclusion chromatography

P-12 *Anikó Kilár*, Ágnes Dörnyei, Béla Kocsis and Ferenc Kilár*
University of Pécs, Pécs, Hungary
Mass spectrometry of bacterial lipid A species

P-13 *Katalin Kispál*, Erzsébet Szász, Sophie Lecomte, Wilmar van Grondelle, Céline Valéry, Bernard Desbat and Sándor Kunsági-Máté*
University of Pécs, Pécs, Hungary
Concentration dependent kinetics of self association of somatostatin molecules

P-14 *András Kiss*, Zsolt Bánfai, Karel Lacina, Jirí Zeravik, Petr Skládal, Géza Nagy*
University of Pécs, Pécs, Hungary
Selective amperometric determination of pyrocatechol and phenol in wines with flow-injection analysis

P-15 *András Kiss* and Géza Nagy*
University of Pécs, Pécs, Hungary
CO_2 partial pressure imaging in gas phase with Scanning Electrochemical Microscopy (SECM)

P-16 *László Kiss* and Barna Kovács*
DDKKK Innovation Non-profit Inc., Pécs, Hungary
Determination of gaseous H_2S using carbon fiber microwire electrode pair

P-17 *Sándor Kunsági-Máté* and Koichi Iwata*
University of Pécs, Pécs, Hungary
Effect of solvation shell composition on the weak molecular interactions of aromatic moieties

P-18 *Karel Lacina*, András Kiss, Zsolt Bánfai, Jirí Žeravík, Petr Skládal and Géza Nagy*
Masaryk University, Brno, Czech Republic
Four-channel enzyme biosensor for determination of phenolic compounds in wine

P-19 *Nándor Lambert* and Ferenc Kilár*
University of Pécs, Pécs, Hungary
Analysis of inorganic anions in mineral waters with isotachophoresis

P-20 *Sophie Lecomte*, Wilmar van Grondelle, Céline Valéry, Katalin Kispál, Erzsébet Szász, Bernard Desbat and Sándor Kunsági-Máté*
CNRS-Université Bordeaux, Bordeaux, France
Self association process of somatostatin

P-21 *Gábor Maász, László Márk**
University of Pécs, Pécs, Hungary
Mass spectrometric analysis of mycolic acids as mycobacterial lipid biomarkers

P-22 *Lilla Makszin*, Anikó Kilár, Péter Felső, Béla Kocsis, Ferenc Kilár*
University of Pécs, Pécs, Hungary
Endotoxins with highly sensitive microfluidic CE analysis

P-23 *Lívia Nagy*, Tünde Angyal, Matsumoto Akiko, Jan Pribyl, Petr Skladal, Géza Nagy*
University of Pécs, Pécs, Hungary
Electrochemical sensor development for measurement of ROS in ethanol induced stress

P-24 *Livia Nagy*, Zsolt Bánfai and Géza Nagy*
University of Pécs, Pécs, Hungary
Amperometric cell for enzyme activity measurement

P-25 *Lívia Nagy*, Szabina Geges and Géza Nagy*
University of Pécs, Pécs, Hungary
Heavy metal analysis in ash of biomass

P-26 *Csilla Páger*, Andrea Vargová and Ferenc Kilár*
University of Pécs, Pécs, Hungary
Effect of the pH of the anolyte and catholyte solutions in CIEF separation with sequential injection set-up

P-27 *Beáta Peles-Lemli*, Walter M.F. Fabian, László Kollár and Sándor Kunsági-Máté*
University of Pécs, Pécs, Hungary
The adsorption behaviors of aniline on carbon nanotubes

P-28 *Anna Takácsi-Nagy*, Csilla Páger, Ferenc Kilár, Wolfgang Thormann*
University Of Pécs, Pécs, Hungary
Advances of computer modeling in capillary isoelectric focusing

P-29 *Anikó Takátsy*, Katalin Böddi*, Lajos Markó, István Wittmann, Róbert Ohmacht, Günther K. Bonn and Zoltán Szabó*
University of Pécs, Pécs, Hungary
Comparison of newly developed fullerene(C60)-silica and convenient solid phase extraction (SPE) materials for nonglycated and glycated peptides

P-30 *Katalin Tálos*, Edina Ács, Alžbeta Hegedűsova, Tímea Pernyeszi*
University of Pécs, Pécs, Hungary
Effect of surface modification of Saccharomyces cerevisiae onto cadmium adsorption

P-31 *Petr Tůma* and Eva Samcová*
Charles University, Prague 10, Czech Republic
Determination of neutral carbohydrates by capillary electrophoresis with contactless conductivity detection

P-32 *Ágnes Varga*, Javier Izquierdo, Lívia Nagy, Ricardo M. Souto and Géza Nagy*
University of Pécs, Pécs, Hungary
SECM study of corrosion measurement of Zn(II) with ion-selective microelectrodes

P-33 *Ladislav Vaško* and Janka Vašková*
University of Pavol Jozef Šafárik, Košice, Slovak Republic
Relevance of humic acids in food production, prophylaxis and treatment

P-34 *Janka Vašková*, Ladislav Vaško, Juraj Guzy and Pál Perjési*
University of Pavol Jozef Šafárik, Košice, Slovak Republic
Activation of antioxidant defence by selected dimethylaminochalcones in mitochondria

ABSTRACTS

LECTURES L-01 - L-19

L-01

MINIATURIZATION IN BIOANALYSIS

František Foret
Institute of Analytical Chemistry of the ASCR, v.v.i.
Veveri 97, 602 00 Brno, Czech Republic.
foret@iach.cz

With the accelerating progress in biology, medicine and related research fields there is a permanent need for better analytical tools. System miniaturization is often cited as a prerequisite for increased speed of analysis and reduced cost of reagents. Additionally, as the size of the analytical devices shrinks, physicochemical phenomena, unimportant on the macro scale, may become dominant, opening new possibilities for conducting analyses. Such systems, often micromachined by processes common in microelectronics, allow manipulation and detection of extremely small sample amounts including preconcentration, extraction, loading/mixing, and/or separation. Significant improvements can be achieved by using enzymes immobilized on a solid support such as beads or monolithic columns. For ultimate miniaturization the enzyme can be immobilized on the wall of the microchannels or attached to nanoparticles and the whole analytical system integrated using a microfluidic chip designed for direct coupling with mass spectrometry. On the other hand the shrinking size provides also lower sample loading capacity leading to the need of different designs for different types of analyses. Clearly, the single cell analysis will need a different approach than agricultural analysis. While traditional column based analytical systems will dominate the chemical analysis market in the foreseeable future, new tools are under development for expansion into new areas, especially in biology related research. This presentation will describe some of the new technology developments related to miniaturization, mass spectrometry coupling, sample enrichment and detection.

L-02

MECHANISTIC ASPECTS OF ENANTIOSEPARATIONS OF SELECTED CHIRAL DRUGS IN AQUEOUS AND NON-AQUEOUS CAPILLARY ELECTROPHORESIS

Bezhan Chankvetadze

Institute of Physical and Analytical Chemistry and Molecular Recognition and Separation Science Laboratory, School of Exact and Natural Sciences, Tbilisi State University, Chavchavadze Ave 3, 0179 Tbilisi, Georgia, bezhan_chankvetadze@yahoo.com

Capillary electrophoresis (CE) represents one of the major techniques not only for analytical scale enantioseparations but is also a powerful tool for a better understanding of the fine mechanisms of enantioselective intermolecular recognition. The major advantages of CE from the viewpoint of enantioselective molecular recognition studies are the following: 1. CE allows very fast screening of selector-selectand pairs. 2. The high peak efficiency in CE permits to observe enantioselective features in selector-selectand interactions which are invisible by other techniques. 3. A small thermodynamic selectivity of recognition can be transformed into a high separation factor in CE. 4. CE is very flexible for adjustment of enantioseparation.

The major disadvantage of CE for studies of non-covalent intermolecular interactions is that this technique does not provide any direct information regarding the structure of intermolecular diastereomeric associates. The experiments based on the nuclear Overhauser effect (NOE) in nuclear magnetic resonance (NMR) spectroscopy complement CE from this viewpoint very well. In addition, NMR-spectroscopy is very useful technique for determination of stoichiometry and enantioselective binding constants of selector-selectand associates. This presentation summarizes our recent studies on the combined application of CE and NMR methodologies to mechanistic studies of enantioselective selector-selectand interaction in the liquid phase. The methodology is illustrated with the examples including interaction of chiral drugs such as ketoconazole and terconazole [1], propranolol [2], ephedrine, norephedrine and tetrahydrozoline with various cyclodextrins in aqueous and non-aqueous medium.

References

[1] Lomsadze, K., Martinez-Giron, A. B., Castro-Puyana, M., Chankvetadze, L., Crego, A. L., Salgado, A., Marina, M. L., Chankvetadze, B., *Electrophoresis* **30** (2009) 2803-2811.
[2] Servais, A.-C., Rousseau, A., Fillet, M., Lomsadze, K., Salgado, A., Crommen, J., Chankvetadze, B., *Electrophoresis* **31** (2010) 1467-1477.

L-03

THE PROTEOME BUCCANEERS: HOW TO UNEARTH YOUR TREASURE CHEST VIA COMBINATORIAL PEPTIDE LIGAND LIBRARIES

Pier Giorgio Righetti[1]* and Egisto Boschetti[2]

(1) Politecnico di Milano, Dept. Chemistry, Via Mancinelli 7, Milano 20131, Italy, piergiorgio.righetti@polimi.it

(2) Bio Rad Labs., Hercules, CA, USA

Combinatorial peptide ligand libraries (CPLLs), via their ability of "normalizing" the content of any proteome, allow a deep exploration of the low abundance proteome. In the case of cytoplasmic red blood cell (RBC) proteome, we discovered 1578 unique gene products [1]. Such unique list of previously unreported species allowed to unravel the genetic defect of congenital dyserythropoietic anaemia II, a rare RBC disorder [2]. In the case of the haemolymph of Limulus polyphemus (a living fossil, 440 million years old), where barely 10 proteins had been described, CPLLs allowed the discovery of >150 unique gene products, although the number of peptides sequenced would call for a grand total of >1500 proteins [3]. The fact that 90% of them could not be identified suggests that these are ancestral proteins whose sequences cannot be found in any database. In the case of rDNA proteins for human consumption, CPLLs allowed the discovery of trace host proteins undetectable in untreated samples. Data will also be presented on the quantitation of trace fining agents (casein) in white wines, down to barely 1 µg/L [4]. This detection limit is at least two orders of magnitude higher than the one of the ELISA test currently in use, which can barely assess 200 µg casein per litre.

References

[1] 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.

[2] Bianchi, P., Fermo, E., Vercellati, C., Boschetti, C., Barcellini, W., Iurlo, A., Marcello, A.P., Righetti, P.G., Zanella, A.. *Human Mutat*. **30** (2009) 1292-1298.

[3] D'Amato, A., Cereda, A., Bachi, A., Pierce, J.C., Righetti, P.G., *J. Proteome Res*. 2010 Apr 18, PMID: 20397719.

[4] Cereda, A., Kravchuk, A.V., D'Amato, A., Bachi, A., Righetti, P.G., *J. Proteomics* (2010) in press.

L-04

CAPILLARY ELECTROPHORESIS BASED ANALYTICAL AND STRUCTURAL PREDICTION TOOLS FOR GLYCAN ANALYSIS

Stefan Mittermayr[1,2], Jonathan Bones[2], Zoltán Szabó[3], Pauline M. Rudd[2], Barry L. Karger[3] and András Guttman[1,3]*

(1) Horváth Laboratory of Bioseparation Sciences, University of Debrecen, Hungary
(2) Dublin-Oxford Glycobiology Laboratory, University College Dublin, Ireland
(3) Barnett Institute, Northeastern University, Boston, MA

The advent of robust and high resolution capillary electrophoresis (CE) technology enabled to establish the detailed analysis and sequencing of glycan structures released from glycoproteins of biomedical and biotechnology interest. Here we present our first results towards establishing a comprehensive database to support the interpretation and structural assignment of glycan profiles obtained by capillary electrophoresis. The database contains capillary electrophoresis analytical parameters and migration positions defined by their glucose unit values (GU) for over 50 APTS labeled N-glycan structures together with experimental and predicted products of their exoglycosidase digestions. The database enlists the structure, molecular mass and capillary gel electrophoresis migration positions for each glycan entry. These observed parameters reflect the molecular mass and three-dimensional shape of the carbohydrate structures and, therefore, provide information relating to position- and linkage-isomer specificity. This introductory part of our database has been constructed by analyzing N-linked oligosaccharides released by PNGase F digestion from standard glycoproteins. We also propose an algorithm for structural prediction for each detected CE peak, which automatically assigns structures based on the GU unit value. In ambiguous cases it can be used in combination with exoglycosidase digestions. Similar to its HPLC counterpart (GlycoBase), this novel database tool holds the promise to further basic glycomics research by enabling quantitative high-throughput analysis of low concentrations of glycans released from glycoproteins.

L-05

INTEGRATION OF GROUND AEROGEL PARTICLES AS CHROMATOGRAPHIC STATIONARY PHASE INTO MICROCHIP

Attila Gáspár*, Andrea Nagy, István Lázár

Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4010 Debrecen, POB. 21., Hungary

gaspara@tigris.unideb.hu

C16 modified and ground silica aerogel particles in submicrometer size, as a new type of stationary phase was prepared and integrated in polydimetilsiloxane (PDMS) microchip. The aerogel particles were packed into the microfluidic channel using a simple, reproducable procedure, which does not require any special frit or fabrication step to retain the particles. The extreme high porosity of aerogels is advantageous not only for the effectiveness of chromatography, but it provides smaller hydraulytic resistance against transportation of liquids through the packing. This latter characteristic is particularly critical in PDMS chips, where only a few bar pressure can be applied at the microfluidic channels to prevent the destruction of the chip. Through aerogel packing higher flow rate of liquid can be achieved, which makes possible faster analysis than with conventional chromatographic packings.

Food dyes as test components could be separated within 10 s. A 50-fold preconcentration could be achieved by retaining 100 nL volume of sample on the packing and elution with metanol.

L-06

MINIATURIZED TECHNOLOGIES IN BIOCHEMICAL ANALYSIS

Johan Roeraade
Royal Institute of Technology, School of Chemical Science and Engineering, Department of Analytical Chemistry, SE-100 44 Stockholm, Sweden
jroe@kth.se

The interest in microchip systems for (bio)chemical analysis is enormous and has been growing exponentially during the last two decades. The outstanding and amazing advances in micro-electronics and associated computer systems are often referred to as an example, where it is repeatedly stated that " the lab on a chip" will be next in line for a similar success. Yet, in reality, there is still a frenetic search for "the killer application". What is the reason for this discrepancy?

In this lecture, some of the fundamental drawbacks of chip-based systems will be discussed – but also some of the great potentials of miniaturization will be outlined – and it will be shown that chip-based systems can certainly be, but are not always the only way, to go !

Examples will be given from ultra-miniaturized systems for separation of biomolecules as well as from mass spectrometry. Mass spectrometry has already been indispensable for a long time in application fields like proteomics, drug development etc, but the technique can still become far more important. If the sensitivity of mass spectrometry could be increased by some orders of magnitude, it may become a serious competitor to immunoassay technologies. Recently, we demonstrated that significant improvements in terms of sensitivity can be obtained with miniaturized inlet systems. Using microchip-based targets for MALDI-TOF MS we reached a detection limit down to a low zeptomole level, and with pico- electrospray-MS we were able to obtain useful spectra from discrete samples down to about 10 attomole. The new technologies will be outlined and some operation criteria will be discussed.

L-07

THE REQUIREMENTS FOR HIGH RESOLUTION AND HIGH REPRODUCIBILITY IN CAPILLARY ELECTROPHORESIS

Stellan Hjertén

Institute of Biochemistry and Organic Chemistry, Faculty of Science, BMC, University of Uppsala, Box 576, SE-75123 Uppsala, Sweden

stellan.hjerten@biorg.uu.se

This meeting is devoted to bioanalysis, which means that one can expect many presentations based on studies in which commercial instruments for electrophoresis, chromatography and mass spectrometry have been used. The apparatus is often fully automated which has many advantages, but contributes to stereotype experiments. However, there is still room for modifications. In this lecture I will confine myself to capillary electrophoresis and give two examples (and still more in future presentations).

1) The application of gels of methoxylated agarose as a complement to polyacrylamide gels for electrophoretic molecular-sieving of macromolecules:

a) In sharp contrast to conventional polyacrylamide gels they have a low UV absorption at 210 nm and therefore permit quantitative determinations of low protein and DNA concentrations without a pre-staining. A correct determination of the amount of the sample in a zone requires much attention, as always.

b) The methoxylated agarose can be used for automated analyses.

c) Scanning of the gel following an electrophoretic analysis: With the voltage switched off the gel is forced out of the capillary, using a syringe pump. This detection method gives the true separation pattern, while the conventional, UV on-line detection technique gives an apparent picture.

2) A 1000-fold on-tube concentration of proteins by displacement electrophoresis in combination with a hydrodynamic counter flow.

Reference

S. Hjertén, D. Eaker, K. Elenbring, C. Ericson, K. Kubo, J.-L. Liao, C.-M. Zeng, P.-A. Lidström, C. Lindh, A. Palm, T. Srichaiyo, L. Valtcheva and R. Zhang., *Jpn. J. Electroph.* **39** (1995) 1-14.

L-08

HEPTAKIS-(2-O-SULFO-3-O-METHYL-6-O-ACETYL)-CYCLOMALTOHEPTAOSE, A SINGLE-ISOMER CHIRAL RESOLVING AGENT FOR ENANTIOMER SEPARATIONS BY CAPILLARY ELECTROPHORESIS

Gyula Vigh* and Edward Tutu

Chemistry Department, Texas A&M University, College Station TX 77842

Our group has developed single-isomer sulfated cyclodextrins (SISCDs) with varied substituents at the C2, C3 and C6 positions of the glucopyranose subunits to provide a variety of intermolecular interactions to facilitate enantiomer separations by CE. The first generation SISCDs carried identical hydroxy, methoxy or acetyl groups at the C2 and C3 positions and sulfo groups at the C6 positions. The second generation SISCDs carried nonidentical groups at the C2 and C3 positions: methoxy groups at the C2, hydroxyl or acetyl at the C3 and sulfo groups at the C6 positions of the glucopyranose subunits. These SISCDs were used for the CE separation of acidic, basic, neutral and ampholytic enantiomers. The trends in the effective mobilities and separation selectivities as a function of the SISCD concentrations followed the prediction of the ionic strength-corrected charged resolving agent migration model.

The first single-isomer, sulfated β-CD that carries the *O*-sulfo group only at the C2 positions, the sodium salt of heptakis(6-*O*-acetyl-3-*O*-methyl-2-*O*-sulfo)-cyclomaltoheptaose has been synthesized by reacting β-CD, sequentially, with dimethyl-t-butylchlorosilane and benzylbromide to form heptakis(6-*O*-dimethyl-*t*-butylsilyl-2-*O*-benzyl)cyclomaltoheptaose. This intermediate was reacted with iodomethane, then the product was selectively deprotected with hydrogen fluoride yielding heptakis(3-*O*-methyl-2-*O*-benzyl)cyclomaltoheptaose. The fourth intermediate was reacted with acetic anhydride, then debenzylated by hydrogen over Pd/charcoal to obtain heptakis(6-*O*-acetyl-3-*O*-methyl)cyclomaltoheptaose. Finally, this intermediate was reacted with the pyridinium complex of sulfur trioxide, followed by sodium hydrogen carbonate to obtain the sodium salt of heptakis(6-*O*-acetyl-3-*O*-methyl-2-*O*-sulfo)cyclomaltoheptaose.

Structural identities of the intermediates and the final product have been determined by 1D and 2D NMR and high resolution MALDI-TOF-MS. HILIC and non-aqueous, gradient reversed-phase HPLC methods have been developed for the determination of their purities, which typically were in excess of 97% mol/mol. The new SISCD has been used as chiral resolving agent for the CE separation of a set of weak base and nonionic enantiomers at pH 2.5.

L-09

DETERMINATION OF ALCOHOL MARKERS IN BODY FLUIDS BY CE

Wolfgang Thormann
Department of Clinical Pharmacology and Visceral Research, University of Bern, Murtenstrasse 35, 3010 Bern, Switzerland
wolfgang.thormann@ikp.unibe.ch

The determination of markers linked to alcohol consumption and abuse is a challenging task. Alcohol intake leads to the formation of direct metabolites, such as ethyl glucuronide and ethyl sulfate, and ethanol induced changes of the levels of endogenous compounds, including liver enzymes, carbohydrate deficient transferrin (CDT) and the urinary ratio of 5-hydroxytryptophol and 5-hyroxyindolacetic acid. This presentation will provide a brief overview on the use of capillary electrophoresis (CE) for the determination of alcohol markers, including ethyl glucuronide, ethyl sulfate and CDT, in body fluids [1-5]. The focus will be on resolution, detection, precision, throughput and quality control. Capillary electrophoresis is thereby shown to be an effective and economical approach for determination of these analytes in clinical and forensic analysis.

References

[1] Lanz, C., Kuhn, M., Deiss, V., Thormann, W., *Electrophoresis* **25** (2004) 2309-2318.
[2] Joneli, J., Lanz, C., Thormann, W., *J. Chromatogr. A* **1130** (2006) 272-280.
[3] Marti, U., Joneli, J., Caslavska, J., Thormann, W., *J. Sep. Sci.* **31** (2008) 3079-3087.
[4] Jung, B., Caslavska, J., Thormann, W., *J. Chromatogr. A* **1206** (2008) 26-32.
[5] Jung, B., Caslavska, J., Thormann, W., *J. Sep. Sci.* **32** (2009) 3497-3506.

L-10

ADVANCES IN BIOANALYTICAL APPLICATION OF NANOTECHNOLOGIES

Karel Kleparnik
Institute of Analytical Chemistry Czech Academy of Sciences
Veveri 97, 61142 Brno, Czech Republic
klep@iach.cz

The recent development of nanotechnologies brings new extraordinary phenomena into the practice of analytical chemistry. The combination of specific luminescent probes including immunoluminescent probes with highly fluorescent labels and an advanced optical instrumentation allows even the single molecule probing of individual cells. The application of highly stable semiconductor quantum dots in laser-induced luminescence detection and surface enhanced Raman scattering, are two examples of methods where the advantageous properties of nanomaterials are taken. While in luminescence spectrometry, a luminophor of relatively high quantum efficiency must be conjugated with a high-affinity selector, e.g., an antibody, in surface enhanced Raman scattering, 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 characterization and application of nanoparticles in analytical and diagnostic practice. The examples of the analyses of quantum dots and their conjugates with biologically important molecules by capillary electrophoresis and detection of important molecules and receptors in cells by high sensitivity luminescence microscopy will be demonstrated. Discussed will be the potential of label-free detection and molecular identification by capillary electrophoresis with the detection of surface enhanced Raman scattering.

Acknowledgement
This research was supported by grant of the Grant Agency of the Czech Republic 203/08/1680, grant of the Grant Agency of the Czech Academy of Sciences KAN400310651 and institute research plan AV0Z40310501.

L-11

NEW TRENDS IN DESIGN OF CHIRAL SENSORS AND BIOSENSORS

Marek Trojanowicz

Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland, trojan@chem.uw.edu.pl

The chiral separation and selective determination of particular optical isomer of a given compound is a challenging task for contemporary chemical analysis. It is a problem of great importance for many areas of scientific research, as well as for various routine analytical applications. Most of biochemical systems functioning in living organisms involve chiral interactions resulting from different stereochemistry of numerous biologically active compounds. Reactions involving enzymes, functioning of transmitters and receptors, numerous metabolic pathways are most often stereoselective. Enantiomeric purity of various com-pounds is important in stereospecific synthesis, production of pharmaceuticals, pesticides and some food additives, where only one enantiomer may interact satisfactorily.

Chiral analysis is at present predominated by high performance chromatographic and electromigration methods that require advanced instrumentation and complex optimization of analytical procedures. Separations are based on the use of chiral stationary phases or chiral selectors, and their interactions with separated species may involve multiple hydrogen bonds, π-π interactions, dipole-dipole and ionic interactions. The same interactions might be employed in design of chemical or biochemical enantioselective sensors, hence determination of particular enantiomer with the use of enantioselective sensor or biosensor might be a potential alternative for numerous routine analytical applications might. Successful design of such sensors, however, is a great challenge compared *e.g.* to chromatographic methods, where -separation of optical isomers is a result of multiple unit operations of separation in each measurement.

The search for such electrochemical sensors can be dated back to late 1970-ties, when enantioselectivity of some potentiometric ion-selective electrodes with neutral carriers in the plasticized membranes was observed. Generally, however, it did not attract desired attention in development of electrochemical sensors. Compounds employed as chiral selectors in separation methods involve cyclodextrins, natural polysaccharides and their derivatives, macrocyclic antibiotics, chiral crown ethers, calixarenes, proteins, chiral surfactants, and ligand-exchange complexes. They can be employed also as potential ionophores in electro-chemical sensors.

As chemically active species providing enantioselectivity of sensors can be also used ion-channels in ion-selective electrodes, enzymes for determination of substrates or inhibitors in amperometric biosensors, doped conducting polymers in potentiometric or piezoelectric sensors, enantioselective antibodies in piezoelectric and micro-electromechanical devices, or molecular imprinted polymers in potentiometric sensing. The possibility of the use of kinetic effects in chiral recognition is still an interesting field for investigation with potential application e.g. in fast flow-injection measurements based on recording of transient signals.

L-12

LIQUID PHASE SEPARATION STRATEGIES FOR IN-DEPTH PROTEOMICS PROFILING

Ziad El Rassi* and Subhashini Selvaraju

Department of Chemistry, Oklahoma State University, Stillwater, OK 74078, USA

elrassi@okstate.edu

In this talk, we will first describe integrated fluidic platforms composed of tandem affinity columns for the depletion of high abundance proteins from human serum and on-line fractionation/concentration of medium and low abundance proteins by tandem immobilized metal-ion affinity chromatography (IMAC) columns and reversed phase (RP) column for in-depth proteomics analysis. The depletion columns were based on monolithic polymethacrylate with surface immobilized protein A, protein G´, and antibodies for depleting the top 8 high abundance proteins. The IMAC fractionation/concentration columns consisted of monolithic stationary phases with surface bound iminodiacetic acid (IDA) chelated with Zn^{2+}, Ni^{2+} and Cu^{2+} while the RP column was packed with nonpolar polymer beads. The integrated multicolumn fluidic platform was very effective in reducing simultaneously both the dynamic range differences among the protein constituents of serum and the complexity of the proteomics samples, thus facilitating the in-depth proteomics analysis by two-dimensional electrophoresis (2-DE) followed by MALDI-TOF and LC-MS/MS. In fact, the number of detected spots was ~ 1450 using SYPRO^{R} fluorescent stain from which 384 spots were subsequently detected by Coomassie Blue. Since the investigation was simply a proof of concept, 295 proteins were readily identified in some selected spots by MALDI-TOF and LC-MS/MS.

In another platform, the peptide beads library technology (Proteominer), which in principle allows the enrichment of proteins to the same concentration level (i.e., protein equalizer) regardless of the original protein abundance in a given biological fluid, was integrated with the same tandem IMAC columns and RP column described above. The 4 fractions from the tandem columns were subsequently fractionated by two-dimensional gel electrophoresis (2-DE). The four fractions from the tandem columns were either solution digested and analyzed by LC-MS/MS or the 2-DE spots were excised and tryptically digested and subsequently identified by LC-MS/MS. The strategy of subsequent fractionation on 4 tandem columns after equalization allowed the identification of more proteins than simply using the equalization by Proteominer. These data will be discussed in this oral presentation. In addition, the effectiveness of the Proteominer approach will be compared to the depletion approach in terms of in-depth proteomics profiling.

L-13

COMPARISON OF DIFFERENT PROCEDURES TO EXTRACT DNA FROM LONG-TERM PRESERVED FORMALIN FIXED TISSUES FOR PCR AMPLIFICATION

Stefan Paireder[1], Josef Bailer[2], Wolfgang Werther[2], Erich Schmid[2], Beatrix Patzak[2], Margit Cichna-Markl[1]*

(1) Department of Analytical Chemistry, University of Vienna, Währinger Str. 38, 1090 Vienna, Austria; margit.cichna@univie.ac.at

(2) Federal Pathologic-anatomical Museum Vienna, Spitalgasse 2A, 1090 Vienna, Austria

Extraction of DNA from formalin fixed tissues is known to be a challenging task. Since formalin fixation leads to covalent protein-nucleic acid cross links standard DNA extraction protocols generally yield low quantities of highly fragmented DNA which e.g. cannot be amplified by the polymerase chain reaction (PCR). Special protocols have therefore been developed aiming at extracting high amounts of amplifyable DNA from formalin fixed tissues. However, since the studies published so far focused on DNA extraction from short-termed (several days or months) preserved tissues, we aimed at developing and optimizing a procedure enabling the extraction of amplifyable DNA from long-term preserved formalin fixed tissues. Samples were taken from the Federal Pathologic-anatomical Museum Vienna which owns a large number of preparations of parts of human bodies which had been fixed decades ago. Collections of anatomical preparations constitute a huge pool of "old DNA" and therefore may in future be of interest for carrying out retrospective genetic studies.

For two reasons, we did not start our investigations with the museum samples but with porcine heart samples. First, the sample amount which we could take from the museum preparations was, as a matter of course, severely limited, and second, information on details of the procedure used for fixing the tissues are very scarce. We therefore investigated the applicability of different DNA extraction protocols to porcine heart samples which we had previously fixed with three different methods, e.g. buffered formalin solution, unbuffered formalin solution or a historic method named Kaiserling. The extraction conditions yielding the highest amounts of amplifyable DNA were then applied to six museum samples which according to the records had been fixed about 50 to 120 years ago.

In the lecture the results of our investigations will be presented in detail. In brief, the amount and quality of DNA strongly depended on the extraction conditions. In the case of the older museum samples, a commercially available kit particularly developed for DNA extraction from formalin fixed tissues gave better results than any other extraction procedure tested. However, reproducibility of DNA extraction was generally low. In many cases, multiple experiments had to be carried out to obtain at least one extract with appropriate DNA amount and purity.

L-14

SELECTION OF EXPERIMENTAL ANIMALS FOR PROTEOMIC STUDIES

István Földi[1], Géza Müller[2], Gábor Juhász[1], Tamás Janáky[1]*

(1) Department of Medical Chemistry, Faculty of Medicine, University of Szeged, Dóm tér 8., 6720 Szeged, Hungary, janaky@mdche.szote.u-szeged.hu

(2) EGIS Pharmaceuticals PLC, Bökényföldi út 116, 1165 Budapest, Hungary

In quantitative proteomics the most crucial challenge is to control the variables which can influence the experiment-to-experiment variation. These variables could be derived from the technical noise and the biological variation, from the so called total variance. Minimizing the total variance between samples allows of detecting smaller significant differences. Protein expression studies become more problematic as using in vivo animal models, such as mice strains. In this case the most crucial variables are the genetic homogeneity, gender, age and growth/housing conditions.

In proteomics, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is the method of choice for separating complex mixtures of proteins. 2-DE delivers a map of intact proteins, which reflects changes in protein expression level, isoforms, or post-translational modifications with high reproducibility.

Psychiatric and neurodegenerative diseases are the diseases of brain. Our main research interest is the elucidation of proteomic background of these diseases in order to discover new biomarkers and drug target proteins. The aim of this study was to investigate the effect of genetic variability of the experimental animals on the protein profile of mouse brain. To achieve this goal brain proteome of male NMRI (outbred) and C3H/HEN (inbred) mice (derived from same or different litters, from two generations) were compared by 2D-PAGE. Only the significantly altered spots were accepted as real differences ($p<0.05$; $q<0.05$, power>0.8 and fold change≥ 1.5).

The reproducibility of our 2D-PAGE was charecterized by the technical variance (interassay coefficient: CV%=12.5, n=5) and the total variance (interassay coefficient: CV%=16.5, n=5), which are quite favourable comparing to the literature data. In the groups of mice borned on the same day onto a same or different litters we could not detect any differently expressed protein. Principal component analysis justified our original assumptation that the variance in brain protein profile of littermate mice is smaller than that of the non-littermate animals. Comparing littermate or non-littermate animals, almost the same number of significant differences were detected (6-13) regardless when they have borned. The most striking expression differences could be identified between the two mice strains. No significant differences were detected at two generations of the the same parent mice.

These results suggest that the genetic background has an effect on the varinace of protein expression profiles. Although, the variance is lower using littermate mice or two generations from same parents, the classical experimental design (random selection) is feasible for proteomic experiments, too. Although the growth/housing conditions are same it is advantageous to perform experiment with animals borned within a short period of time. The found significant alterations suggest that differences in the date of birth could generate virtual changes in protein expression, independently of any treatment.

L-15

ENGINEERED LACCASE: ARTIFICIAL ENZYMES WITH NEW PROPERTIES

Yingli Liu, Yasmina Mekmouche, Eloine Npetgat, Viviane Robert, Pierre Rousselot Pailley, Sana Ullah and Thierry Tron*

BiosCiences ISM2, CNRSUMR6263, University of Marseille, FRANCE
thierry.tron@univ-cezanne.fr

Laccases are very well known biocatalysts with great potentials in various industrial processes in particular because of their robustness, high oxidation power and substrate versatility (among other properties). Laccases belong to the Blue Copper Binding Domain (BCBD) family of proteins in which the archetypal member is the electron plant or bacterial electron transfer protein cupredoxin (CUP). In this family, function is modulated by the number of CUP domains, the number and type copper atoms and the fusion to non metalled domains. Taking natural plasticity within the BCBD family as a source of inspiration for the engineering of laccases, we aim at creating artificial objects with original functionalities and/or properties. Examples of efficiently modified enzymes obtained in recombinant expression systems will be presented.

L-16

DIFFICULTIES OF CE-LIF IN THE ANALYSIS OF BIOLOGICAL SAMPLES

Éva Szökő*, Tamás Tábi and Zsolt Wagner

Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Nagyvárad tér 4., 1089 Budapest, Hungary, eva.szoko@net.sote.hu

Main difficulties of analyzing biological samples are the usually limited amount of sample specimens, low analyte concentration, the complex sample matrix, etc. CE with LIF detection is usually used when few microliter microdialysates are to be analyzed. LIF is regarded to provide more selective and sensitive detection compared to UV absorbance. However, as majority of analytes has no intrinsic fluorescence, sample derivatization is required, considerably limiting the expected advantages of the method.

The selectivity is rather impaired when the sample is derivatized, since biofluids contain lots of compounds labeled alongside with the analytes of interest. Interfering peaks also derive from the derivatizing reagent either as decomposition products or in case of fluorofore labels as the high excess of the reagent itself. The concentration of these interfering compounds and side products usually highly exceeds that of the analytes. The impaired selectivity is accompanied by impaired sensitivity as the latter depends on the signal-to-noise ratio rather than the signal itself, thus the reported LOD values for analytes in biological matrix are significantly higher compared to those in aqueous solutions. Analysis of biological samples is further complicated when the composition of the sample matrix varies sample by sample resulting in differences in interfering peaks and/or their relative peak size. The complex samples require carefully designed separation conditions to ensure appropriate resolution and peak capacity. Several buffer additives are commonly used simultaneously, to improve selectivity and widen separation time window. The complicated separation buffer may question the robustness of these methods. The high sensitivity of LIF detection is also limited by the unreliable derivatization reaction at low sample concentration. Sample derivatization at the submicromolar concentration suffers from the slower reaction rate and the consequentially increased competition with hydrolysis reaction of the labeling reagent as well as the adsorption of analyte to the surface of the reaction vessel. The consequence of the incomplete derivatization is the loss of linear correlation between the concentration and peak area or height. Calibration in the range of two to three orders of magnitude concentration can raise a bias because of the overrepresentation of the higher concentration points on the calibration curve, since the regression coefficient is determined by the absolute deviation of the measured data points from the fitted line. Due to this bias accurate determination in the lower concentration part of the calibration range can hardly be performed. In line with these problems, the appropriately determined quantification limits may differ considerably from the limit of detection.

These difficulties will be demonstrated through the example of method development for determination of excitatory amino acid neurotransmitters in microdialysates.

L-17

DEVELOPMENT IN "AIRBORNE CHEMISTRY" & PROTEIN EPITOPE MIP PSP-CEC

Staffan Nilsson

Pure and Applied Biochemistry, Center for Chemistry and Chemical Engineering, Lund University, Staffan.Nilsson@analykem.lu.se

L-18

PERFORMANCE OF MODERN HPLC STATIONARY PHASES IN BIOANALYSIS

Attila Felinger*, Ibolya Kiss and Péter Vajda

Department of Analytical and Environmental Chemistry, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary, felinger@ttk.pte.hu

Novel stationary phases in high-performance liquid chromatography have been developed continuously. During the recent years, porous sub-2-micron packing materials, core-shell materials have been introduced to speed up separations keeping the high efficiency. The novel phases guarantee fast mass transfer kinetics due to the shorter diffusion paths.

Therefore, the study of the details of solute transfer in liquid chromatography is of central interest. We apply the microscopic (or molecular dynamic) model of chromatography to study the reversed phase separation of small and large molecules. The microscopic theory of chromatography describes the evolution of a chromatographic peak as the random migration of the molecules along the column combined with adsorption–desorption processes that occur at random, too.

The molecular dynamic model is rather straightforward to comprehend and it can furnish direct answers when one tries to understand the development of chromatographic peaks. We show that the microscopic model can be rather simply used to estimate the fundamental characteristics of the separation process. We can estimate the rate a molecule is adsorbed on the surface of the stationary phase while it migrates along the column.

We analyze the peak shapes recorded under linear conditions, and can characterize the heterogeneity of the surface of the stationary phase. With a peak shape analysis that is based on the molecular dynamic model of chromatography, we can identify the presence of heterogeneous mass transfer or adsorption kinetics. We can, furthermore calculate the amount of retention due to the individual adsorption sites.

The information obtained on the details of the chromatographic process is utilized to optimize HPLC and LC-MS separation of natural samples. The results of the determination of polyphenolic compounds by liquid chromatography–mass spectrometry in Thymus species are presented. Data on the quantitative determination of atropine and scopolamine in the floral *nectar* of *datura* species are also discussed.

We present results obtained on nonporous, fully porous, as well as on shell particles.

ABSTRACTS

POSTERS P-01 – P-34

(in alphabetical order of the first author)

P-01

ANALYSIS AND STABILITY STUDY OF TEMOZOLOMIDE USING CAPILLARY ELECTROPHORESIS

Melinda Andrási[1]*, Rose Bustos[2], Attila Gáspár[1], Frank A. Gomez[2], Álmos Klekner[3]

(1) Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4010 Debrecen, POB. 21., Hungary, andrasimelinda@freemail.hu

(2) Department of Chemistry and Biochemistry, California State University, Los Angeles, CA 90032-8202, USA

(3) Department of Neurosurgery, University of Debrecen, 4012 Debrecen POB. 7., Hungary

Temozolomide (TMZ) is an oral alkylating agent that readily crosses the blood-brain barrier that can be used for the treatment of malignant brain tumors (e.g. glioblastoma) [1]. TMZ is spontaneously hydrolyzed at physiologic pH to the active component 3-methyl-(triazen-1-yl)imidazole-4-carboxamide (MTIC). MTIC is further hydrolyzed to 5-amino-imidazole-4-carboxamide (AIC) and to methyldiazonium cation which is the active alkylating species.

The applicability of micellar electrokinetic capillary chromatography (MEKC) for the analysis of TMZ and its degradants, MTIC and AIC has been studied. Using short and injection, the analysis of TMZ and its degradants could be performed within 1.2 min. The obtained precision of migration times was better than 1.6 RSD% and the limit of quantitation (LOQ) was 0.31-0.93 µg/ml. The therapeutic concentration of TMZ in blood samples can be determined after direct sample injection and conventional on-capillary UV detection [1]. TMZ is rapidly and completely absorbed after oral administration, maximum plasma concentration occur in 20 minutes (average t_{max}: 0.5-1.5 h). Plasma TMZ concentration declined with a mean elimination half-life of 1.9 hours. The proposed MEKC method was applied to study the stability of TMZ in water and serum at different pH values. It was established that the half-life of the TMZ in vitro serum at room temperature was 33 min, close to the half-life (28 min) obtained in water at pH 7.9.

References

[1] Darkes, M.J.M., Plosker, G.L., Jarvis, B., *Am. J. Cancer* **1** (2002) 55-80.

[2] Andrási, M., Bustos, R., Gáspár, A., Gomez, F.A., Klekner, Á., *J.Chromatogr. B* **878** (2010) 1801-1808.

P-02

DETERMINATION OF CHLORIDE ION BY USING FLUORESCENT NANOBEADS

Katalin Balogh[1]*, Péter Ács[2], Barna Kovács[1,2]

(1) DDKKK Inc., Móra Ferenc u. 72/A 7632 Pécs, Hungary, baloghk@gamma.ttk.pte.hu

(2) Institute of Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6. 7624 Pécs, Hungary, kovacs1@gamma.ttk.pte.hu

Several optical methods are used for ion detection in environmental or in clinical samples [1]. For example colorimetric titration, ion selective electrodes [2] or optical sensors are often used for low chloride ion determination. The dynamic range of optical chloride ion sensors is around in the 0.01-100 mM range. Mainly the sensing principle of optical anion sensors are based on fluorescence quenching of indicators by the anion of interest. Thus, positively charged indicators are often used for the determination of anions [3].

In this work a newly synthesized fluorescein-mercury(I)-nitrate was investigated as promising sensing material for low level chloride ion determination. Spectrophotometric and fluorescence measurements were performed in ethanol/water mixture and in boric acid. In further experiments fluorescein-mercury(I)-nitrate was immobilized in polymeric nanobeads according to the literature [4]. The fluorescence intensity of the indicator containing nanobeads increased in the presence of chloride ion when pure water was used as a solvent. Also significant increase caused by chloride ions was observed when increasing the ionic strength by boric acid (0-8 g/l). The selectivity of indicator containing nanobeads was tested both in water and in boric acid towards different anions such as rodanide, nitrate, sulfate and phosphate by using mixed solution method.

References

[1] García, N.H., Plato, C.F. , Garvin, J.F., *Kidney International* **55** (1999) 321-325.

[2] Pimenta, A.M., Araújo, A.N., Montenegro, M.C.B.S.M., Pasquini, C., Rohwedder, J.J.R., Raimundo Jr., I.M., *J. Pharm. Biomed. Anal.* **36** (2004) 49-55.

[3] Huber, C., Werner,T., Wolfbeis, O.S., Leiner, M.J.P., *Anal. Chim. Acta* **398** (1999) 137-143.

[4] Borisov, S.M., Mayr, T., Klimant, I., *Anal. Chem.* **80** (2008) 573-582.

P-03

EPR STUDIES IN YEAST CELLS: EFFECT OF CAROTENOIDS ON PLASMA MEMBRANE DYNAMICS

Ágnes Blaskó[1]*, József Belágyi[2], József Deli[3], Csaba Vágvölgyi[4] and Miklós Pesti[5]

(1) Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624 Pécs, Hungary, agnes.blasko@aok.pte.hu

(2) Department of Biophysics, Faculty of Medicine, University of Pécs

(3) Department of Biochemistry and Medical Chemistry, Faculty of Medicine, University of Pécs

(4) Department of Microbiology, Faculty of Sciences, University of Szeged

(5) Department of General and Environmental Microbiology, Faculty of Sciences, University of Pécs, P. O. Box 266, H-7601 Pécs, Hungary, pmp@gamma.ttk.pte.hu

The carotenoids present in the yeast *Xanthophylomyces dendrorhous* prevent oxidative damages of the plasma membrane. They have different properties (polar, non-polar) which are built in different way into the plasma membrane, forming differing binding forces. This resulted in different the membrane`s structure and dynamic.

Spin-labelling method was applied to study the various properties of the plasma membrane dynamics of *Xanthophylomyces dendrorhous* mutants in relation to temperature. It has been found that the parental strain *CBS 6938* and the *C31* mutant contained polar carotenoids, such as astaxanthin and cis-astaxanthin, increased the electron paramagnetic resonance (EPR) order parameter and decreased the motional freedom, phase-transition temperature and the flexibility gradient of the alkyl chains of the lipids, as shown with stearic acid spin labels. On the other hand, the non-polar carotenoids, β-cryptoxanthin and β-carotene decreased the EPR order parameter and increased motional freedom, phase-transition temperature and the flexibility gradient of alkyl chains.

References

[1] Niewska, A.W., Draus, J., Subczynski, W.K., *Cell. Mol. Biol. Lett.* **8** (2003) 147-159.
[2] Wisniewska, A.,. Subczynski, W.K., *Biochim. Biophys. Acta* **1368** (1998) 235-246.
[3] Subczynski, W.K., Markowska, E., Sielewiesiuk, J., *Biochim. Biophys. Acta* **1150** (1993) 173-181.
[4] Jezowska, I., Wolak, A., Gruszecki, W.I., Strzalka, K., *Biochim. Biophys. Acta* **1194** (1994) 143-148.
[5] Strazalka, K., Gruszecki, W.I., *Biochim. Biophys. Acta* **1194** (1994) 138-142.
[6] Palagyi, Zs, Linka, T.B., Papp, T., Vágvölgyi, Cs. *Acta Aliment. Hung.* **35** (2006) 223-228.
[7] Sujak, A., Strazalka, K., Gruszecki, W.I., *Chem. Phys. Lipids* **145** (2007) 1-12.
[8] Blasko, A., Belagyi, J., Dergez, T., Deli, J., Papp, G., Papp, T., Vagvolgyi, C., Pesti, M., *Eur Biophys J.* (2008) **37(7)** 1097-104.

P-04

VIDEO-MICROSCOPY CAPILLARY ELECTROPHORESIS SYSTEM

Péter Buzási[1]*, Dávid Szabó[1], Ferenc Kilár[1,2], Béla Kocsis[3]

(1) Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12., H-7624 Pécs, Hungary

(2) Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary

(3) Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624 Pécs, Hungary

The video-microscopy associated electrophoresis system is a simple method developed by us, which uses digital video-technique and computer data analysis for the determination of mobility of species of bacteria and fungi. The purpose is to find a method, which is capable to determine the electrophoretic mobility fast and easy and can differentiate between cell-types. We examined different species of bacteria (*Salmonella minnesota*, *Shigella sonnei*, *Escherichia coli*, *Staphylococcus aureus*) and fungi (*Saccharomyces cerevisae*, *Candida albicans*) in coated and uncoated capillaries. The mobilities of the cells were measured by the "visual" electrophoretic system, and calculated by statistical methods. Synchronization of the cells decreases the variation of the mobilities and makes the reproducibility better. The mobilities, and in some cases the orientation of the cells during migration were observed to be different for the different cell-types.

P-05

USE OF SCANNING ELECTROCHEMICAL MICROSCOPY (SECM) FOR THE LOCAL ANALYSIS OF SURFACE REACTIVITY IN BIOMEDICAL TI20MO ALLOY EXPOSED TO DIFFERENT ELECTROLYTIC ENVIRONMENTS.

G. Ciurescu[1], J. Izquierdo[2], J.J. Santana[2], S. González[2], D. Mareci[1], D. Sutiman[1], R.M. Souto[2]

(1) Technical University ''Gh. Asachi", Bd. Mangeron 71, 700050 Iasi, Romania

(2) Department of Physical Chemistry, University of La Laguna, E-38200 La Laguna, Tenerife, Canary Islands, Spain Fax: +34-922-318002, jsantana@dip.ulpgc.es

Scanning electrochemical microscopy (SECM) [1] is a very useful tool for the local investigation of corrosion processes and reactions [2]. SECM provides *in situ* information, in the micrometric and submicrometric ranges, concerning the topography and the electrochemical reactivity of active surfaces exponed to aqueous electrolytes. This technique consists in rastering a probe in close proximity to a surface (typically equal or below the diameter of the tip), and the response measured at the probe is sensitive to changes in the chemical activity and /or surface topography of the simple.

We report on the application of SECM to characterize the chemical characteristics of a biomedical Ti20Mo alloy exponed to various electrolytes, namely 0.1 M NaCl and Ringer´s physiological solution. The sample was used both unpolarized (i.e. left at its spontaneous open circuit potential in the solution) and under polarization until -0.7 V *vs* OCP. Ferrocene-methanol was added to the electrolytes as redox mediator for SECM operation in the feedback mode. *Z*-approach curves are observed to strongly depend on the electrical state of the metal surface under investigation (cf. Figure 1), as well as on the nature of the test solution. These are indications of changes in the surface reactivity of the sample in both situations.

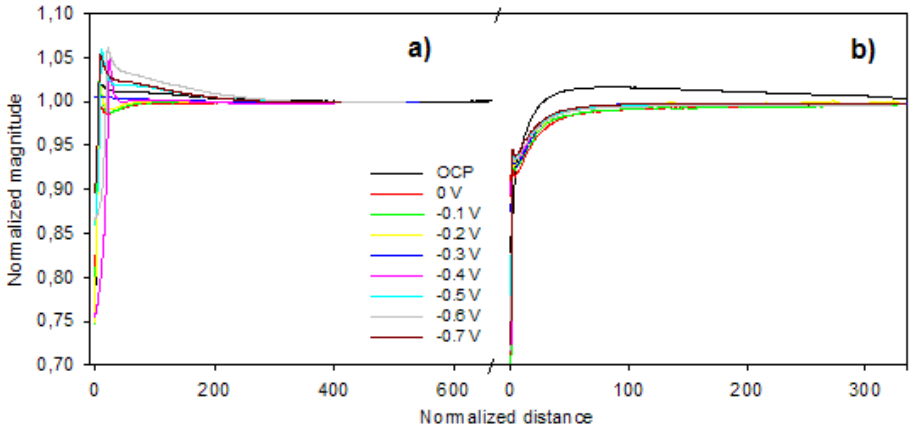


Figure 1: Normalized *Z*-approach curves towards a Ti20Mo sample immersed in 0.1 M NaCl (a) and Ringer (b) solutions. The alloy was left at its spontaneous OCP in the electrolyte. Tip: 10 µm Pt.

References

[1] Bard, A.J.; Fan, F.-R.F.; Kwak, J.; Lev, O. *Analytical Chemistry*, 61 (**1989**), 132-138.

[2] Niu, L.; Yin, Y.; Lu, M.; Qin, R.; Chen, S. *Journal of Materials Science*, 44 (2009) 4511-4521.

P-06

THE DEVELOPMENT OF INVERSE VOLTAMMETRIC METHOD FOR ESTIMATION FOR HEAVY METAL CONTENT IN TORTUOUS ENVIRONMENTAL MEDIA

Zsuzsanna Czibulya[1]*, Lívia Nagy[2], Ágnes Németh[2], Géza Nagy[2]

(1) SCIENCE, Please! Projektiroda, Szántó Kovács János u. 1/B. 7633 Pécs, Hungary
czibulya@gamma.ttk.pte.hu

(2) Department for General and Physical Chemistry, Faculty of Sciences, University of Pécs, Ifjúság u. 6., 7624 Pécs, Hungary

Inverse voltammetric methods are among the analytical measuring techniques providing the lowest limit of detection. Using appropriate accumulation and detection program heavy metal concentrations can be measured in nanomolar ranges with acceptable accuracy and precision. In case of certain environmental or biologic samples it is advantageous if the analysis is carried out in the spot. If no sampling, background electrolyte adding, stirring, purging steps are involved then low level heavy metal concentration with good spatial resolution can be measured avoiding contamination from chemicals and laboratory dishes. In this way using ultra micro carbon fiber electrodes inverse voltammetric measurements can be carried out in living tissues of anesthetized animals and plants, or in humid soil samples. However to be able to measure low level heavy metal concentration inside these complex matrices special problems have to be solved. As it is obvious in these matrices no stirring can be applied, and the diffusion toward the electrode during accumulation step is heavily influenced by tortuousity, by decreased fluid volume ratio and matrix particle - sample ion interaction. In our recent work in situ analytical methods have been investigated in order to work out inverse voltammetric methods for analysis of low level heavy metal concentrations in tortuous matrices. In these work bismuth film coated ultra micro working electrodes are used. The bismuth film is electrochemically formed in an external cell. Owing to the small size of the tip no stirring is needed in the accumulation step. For comparison measurements were carried out with Glassy Carbon electrode and carbon paste electrode. For detection DPV and SWV methods with different parameters were employed. The square wave voltammetric detection was found superior. In our presentation the preparation procedure of the bismuth film coated electrode will be presented. Detailed description of the results obtained with different measuring parameters in soil and plant tissue measurements will be given. Lead and cadmium ions were employed as analytes.

References

[1] Pinamontí, F., Stringari, G., Gasperi, F., Zorzi, G., *Resources, Conservation, and Recycling* **21** (1997) 129-143.
[2] Singh, R.P., Agrawal, M., *Chemosphere*, **67** (2007) 2229-2240.
[3] Xu, H., Zeng, L., Huang, D., Xian, Y., Jin, L., *Food Chemistry* **109** (2008) 834-839.
[4] Farghaly, O. A., Ghandour, M.A., *Environmental Research* **97** (2005) 229-235.

P-07

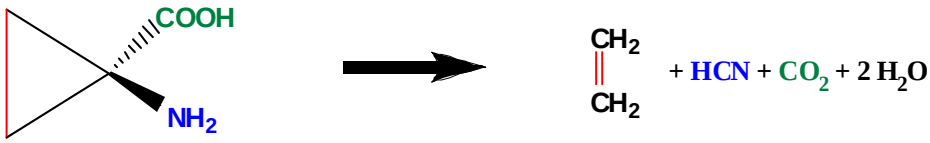
ACC OXIDASE: CHARACTERIZATION AND INACTIVATION MECHANISM

N. El Bakkali-Tahéri*, L. Brisson, V. Robert, M. Réglier, E.-H. Ajandouz and A. J. Simaan

ISM2-UMR 6263 BiosCiences, Marseille, France

nadia.ebt@free.fr

The 1-AminoCyclopropane Carboxylic acid oxidase (ACC Oxidase, ACCO) catalyzes the formation of the plant hormone, ethylene. ACCO catalyzes the oxidation of ACC into ethylene in the presence of dioxygen and two electrons (provided in vitro by ascorbate).



Reaction catalysed by ACC

The crystal structure of ACCO was obtained in 2004 and the active site is composed of a non-heme iron(II) coordinated by a 2His-1Asp facial triad. Given the importance of ethylene in plants, studies on ACCO, as well as on the other enzymes involved in its biosynthesis, have attracted much attention in the past two decades. However, many questions remain unanswered concerning the role of the different cofactors, the catalytic mechanism and the intriguing inactivation processes. ACCO is indeed known for its instability in vitro which could be related to the catalytic cycle.

Using an interdisciplinary approach ranging from kinetic analysis and electrophoresis to spectroscopy, we aim at better understanding the modifications of the enzyme and its inactivation mechanisms.

P-08

BIOSORPTION OF LEAD(II) IONS BY PRETREATED BIOMASS OF *PHANEROCHAETE CHRYSOSPORIUM*

Viktor Farkas[1]*, Krisztina Deák[1], Alžbeta Hegedűsova[2], Tímea Pernyeszi[1]

(1) Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., H-7624 Pécs, Hungary, ptimea@ttk.pte.hu

(2) Department of Chemistry, Faculty of Natural Sciences, Constantine the Philosopher University, Tr. A. Hlinku 1, SK-949 01, Nitra, Slovakia

Heavy metals are discharged from various industries such as electroplating, metal finishing, textile, storage batteries, mining ceramic and glass. As they pose serious environmental problems and are dangerous to human health, considerable attention has been given to the methods for their removal from industrial wastewaters. At present, many technologies, such as sulfuration method, electrolyse, membrane and ion-exchange process, can be used for the treatment of wastewater polluted by heavy metals. However, these methods are less effective and more expensive when heavy metal concentration in the wastewater is low, and some of them are easy to cause the second pollution. Removal of heavy metals by biosorption has many advantages, such as fast adsorption speed, removing heavy metal ions selectively under low concentration, high adsorption efficiency, wide range of pH and temperature, less investment and running cost, in addition, some heavy metals can be recovered.

Phanerochaete chrysosporium is a well-known white-rot fungus and it has a strong ability to degrade various xenobiotics and axist in bleaching effluents from pulp and paper mills. It could also be used to remove heavy metals from wastewaters by adsorbing the metals on its mycelium. The treatment of native biomass improves its biosorption capacity, changes the possible binding groups. In this study the biosorption of heavy metals from aqueous solution on non-living mycelial pellets of *Phanerochaete chrysosporium* treated with caustic, heat and ethanol was studied using batch technique with respect to initial pH value, initial concentration and biomass dosage. *Phanerochaete chrysosporium* was grown in a liquid medium containing mineral and vitamin materials with complex composition. The maximal adsorption capacity for lead removal was determined at pH 6. The kinetics of lead(II) removal and the relevance of adsorption isotherms for characterization of uptake were examined. The biosorption process followed pseudo-second order kinetics. The adsorption data of heavy metals on the blank beads and treated biomass could be well described with Freundlich and Langmuir isotherm equation as well. The biomass of *P. chrysosporium* treated with ethanol revealed that it was mechanical stable and had increased adsorption capacity compared to caustic, heat and untreated cells.

Acknowledgement

Timea Pernyeszi and Alžbeta Hegedűsova gratefully acknowledge the support for this research from the Hungarian-Slovak Intergovernmental & Cooperation Programme (APVV FK-HU 0018-08, SK 18/2008) between the University of Pécs and the Constantine the Philosopher University for 2009-2011.

P-09

PHENOL AND LEAD ADSORPTION ON BENTONITE AND MODIFIED BENTONITE BY BENZYLDODECYLDIMETHYLAMMONIUM BROMIDE IN AQUEOUS SOLUTION

Krisztina Honfi[1]*, Rita Szabó[1], Valéria Hodován[1], Imre Dékány[2], Tímea Pernyeszi[1]

(1) Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., H-7624 Pécs, Hungary

(2) Department of Physical Chemistry and Material Science, Faculty of Science and Informatics, University of Szeged, Aradi Vértanúk tere 1, H-6720 Szeged, Hungary

Clays or organoclays have been used as barrier to prevent the transport of hazardous contaminants in landfills. However, clays are known to effectively sorb mostly inorganic contaminants, while organoclays are mainly used for organic contaminants. Since the organoclays are basically clay particles modified with cationic surfactants, there might exist an optimal coverage of cationic surfactant on the clay particles to sorb both inorganic and organic contaminants. In order to determine the optimal mass of cationic surfactants on the bentonite, bentonites were treated with various ratios of benzyldodecyldimethylammonium bromide (BDDDMA). Phenol and lead were selected as representative contaminants. Sorption kinetics and isotherms of contaminants by organobentonites was selectively studied in the function of surface coverage. When either phenol or lead exists as a single contaminant, phenol sorption increased with increasing surface coverage, and lead sorption decreased with increasing BDDDMA to bentonite ratios. Sorption of phenol was a function of BDDDMA coverage on the bentonites, while lead sorption was much more influenced by the initial lead concentration than the mass of surfactants added to the bentonites.

Reference

[1] Senturk, H.B., Ozdes, D.Gundogdu, A., Duran, C., Soylak, M., *Journal of Hazardous Materials* **172** (2009) 353-362.

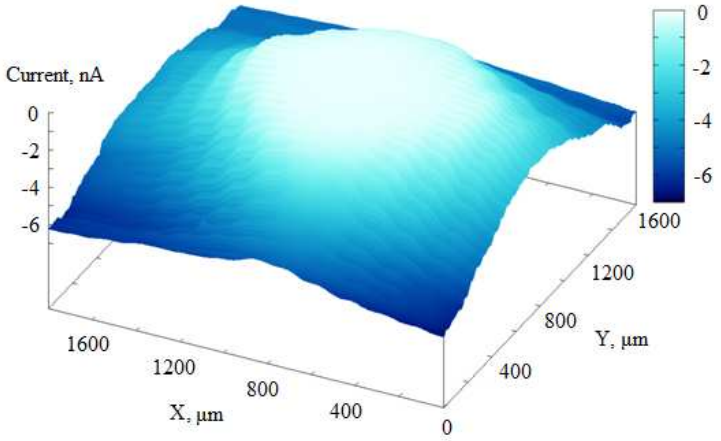
P-10

DETECTION OF ELECTROCHEMICALLY ACTIVE SPECIES BEING CONSUMED AND PRODUCED IN METALS BY SCANNING ELECTROCHEMICAL MICROSCOPY

Javier Izquierdo[1], Lívia Nagy[2], Géza Nagy[2], Ricardo M. Souto[1]

(1) University of La Laguna, Dpto. Química Física, 38200 La Laguna (Tenerife), Spain
(2) Department of General and Physical Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary

As results of heterogeneous reactions local concentration profiles of the reacting or evolving species are generated in the fluid phase. In certain reactions oxygen is directly reduced forming H_2O_2, and generating local increase of the pH. For obtaining detailed information about these local processes appropriate analytical method is needed. Scanning Electrochemical Microscopy (SECM) is a powerful technique for monitoring local evolution of species. Its applicability with amperometric, as well as with potentiometric measuring mode [1, 2] have been well proved in investigation of biological and other kind of processes [3]. Recently we became interested in investigating electrochemical and microbial corrosion reactions. We use electrochemical microscopy and work out special measuring tips and cells. In this work, to be presented, oxygen and H_2O_2 concentrations, as well as pH modifications, have been analyzed above a metal target using SECM technique. The measuring techniques employed will be described. Concentration profiles over corroding surfaces gathered will be presented. Some details of the processes as well as mass flow of materials involved in the reaction in different conditions will be discussed.



Current obtained scanning above Fe disc (the wire was connected with Zn sacrificing metal).
OD 25 µm Pt tip, potential: -700 mV vs. Ag/AgCl,KCl (3 M)

References

[1] Nagy L., Nagy G. *Fresenius J. Anal. Chem.* **366** (2000) 735-744
[2] Nagy L., Nagy G. *Analytical Letters* **40** (2007) 3-38
[3] Simões A.M., Bastos A.C., Ferreira M.G., González-García Y., González S., Souto R.M. *Corrosion Science* **49** (2007) 726-739

P-11

STATIONARY PHASE CHARACTERISATION WITH TOTAL PORE BLOCKING AND INVERSE SIZE EXCLUSION CHROMATOGRAPHY

Silvia Jakabová[1,2]*and Attila Felinger[1]

(1) Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság u. 6, 7624 Pécs, Hungary

(2) Department of Chemistry, Faculty of Natural Sciences, Constantine the Philosopher University in Nitra, Tr. A. Hlinku 1, 949 01 Nitra, Slovakia, sjakabova@ukf.sk

The total volume of a particle-packed RPLC column can be written as the sum of three contributions: the interparticle, interstitial, or external pore volume; the intraparticle or internal pore volume; and the inaccessible volume which can be split into the stationary-phase solid volume, the closed-pore volume, and the volume of the bonded chains [1].

Inverse size-exclusion chromatography (ISEC) presents a common method for investigation of properties of stationary phase. In ISEC method, based on mechanical exclusion, solutions of known polymeric samples are injected into a column packed with an unknown adsorbent, and the retention behavior of these solutes can be correlated with the pore size distribution of the packing material [1].

Total pore blocking (TPB) method is considered as an alternative method to determine the interstitial void volume and the external porosity inside the column. The method is based on measuring of elution time of tracers with small molecular weigh (e.g. uracil or thiourea) after filling the internal micro and mesopores of packing material with non-polar solvent, which is immiscible with polar eluent, used as a mobile phase [2, 3].

In this work we focus on the characterisation of selected properties of stationary phase of two reversed-phase columns that differ in packing material.

Luna 2.5u C18(2)-HST column (100×3.0mm, 2.5µm, Phenomenex, USA), packed with totally porous particles, and the Kinetex 2.6u C18 100A column (100×3.0mm, 2.6µm, Phenomenex, Germany), filled with superficially porous particles, were investigated on UFLC equipment (Prominence, Shimadzu, Japan) by the mentioned methods. The results of the investigations are presented.

Acknowledgement

The work was supported by the project SROP-4.2.2/08/1/2008-0011.

References

[1] Guan, H., Guiochon, G., *J. Chromatogr. A* **731** (1996) 27.
[2] Cabooter, D., Lumen, F., Sandra, P., Desmet, G., *J. Chromatogr. A* **1157** (2007) 131–141.
[3] Liekens, A., Cabooter, D., Denayer, J., Desmet, G., *J. Chromatogr. A* (2010) in press.

P-12

MASS SPECTROMETRY OF BACTERIAL LIPID A SPECIES

Anikó Kilár[1,2]*, Ágnes Dörnyei[1,3], Béla Kocsis[2], Ferenc Kilár[1,3]

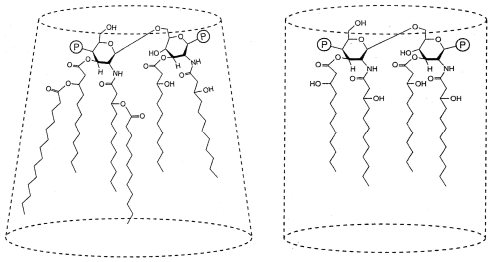
(1) Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary, aniko.kilar@aok.pte.hu

(2) Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624 Pécs, Hungary

(3) Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624 Pécs, Hungary

Lipid A is the toxic part of the bacterial lipopolysaccharides (LPSs or endotoxins), which are found in the outer membrane of all Gram-negative bacteria. The presence of Lipid A or whole LPS in the bloodstream can cause violent pathophysiological reactions, such as high fever, shock or even death.

A Lipid A molecule usually consists of β-(1'→6)-linked D-glucosamine (GlcN) disaccharide backbone bis-phosphorylated at C1 and C4' positions and acylated at 3,3'-hydroxyl and 2,2'-amino groups with β-hydroxylated fatty acids (primary fatty acids), of which two (or three) are further acylated at C3 position with (mostly) nonhydroxylated fatty acids (secondary fatty acids). Lipid A is usually characterized by a heterogeneity mainly due to the type, number, and position of the fatty acids, and by the variability of phosphate substitution. The heterogeneity strongly influences the toxicity properties of the molecule.



Toxic Inactive

Several mass spectrometry techniques are exploited to gain information about the heterogeneity, *i.e.*, the number of different species of the Lipid A families and distribution of the fatty acids, as well as the phosphorylation pattern on each glucosamine unit.

In this study, different Lipid A-s extracted from *Escherichia coli*, *Proteus morganii*, *Salmonella urbana* and *Shigella sonnei* strains have been analyzed by MALDI-TOF and ESI-ion trap mass spectrometers, and their structural diversity is described.

Acknowledgements

The work was supported by the grants GVOP-3.2.1-0168, OTKA-NKTH-NI-68863. Á.D. acknowledges the support of the János Bolyai Research Scholarship (Hungarian Academy of Sciences).

P-13

CONCENTRATION DEPENDENT KINETICS OF SELF ASSOCIATION OF SOMATOSTATIN MOLECULES

Katalin Kispál[1,2]*, Erzsébet Szász[1], Sophie Lecomte[2], Wilmar van Grondelle[3], Céline Valéry[3], Bernard Desbat[2] and Sándor Kunsági-Máté[1]

(1) Department of General and Physical Chemistry, University of Pécs, Ifjusag 6., H-7624 Pécs, Hungary. kunsagi@gamma.ttk.pte.hu

(2) CBMN, UMR 5248, CNRS-Université Bordeaux 1-ENITAB, IECB, Pessac, France. s.lecomte@cbmn.u-bordeaux.f

(3) Ipsen Pharma, Sant Feliu de Llobregat, Barcelona, Spain.

The small, cyclic neuropeptide hormone is known to spontaneously self-assemble into stable liquid crystalline amyloid-like nanofibrils under mild and non-denaturing conditions [1]. Recent works showed that the Somatostatin aromatic residues were proposed to play a role in the self-assembly process [2,3]. Due to the considerable photo-luminescence (PL) of the aromatic moieties of the three amino acids (phenylalanine, tyrosine and tryptophan) [4] the formation of π-π interactions can be followed by PL methods (Figure 1). Furthermore, the anisotropy decay or PL polarization measurements reflect the formation of larger molecular association.

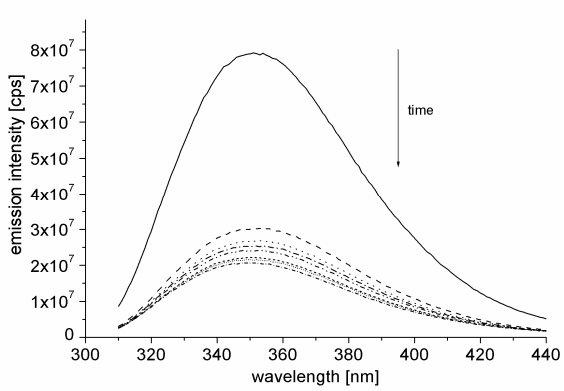


Figure 1 : Decrease of PL intensity during self-organization of the Somatostatine

The photoluminescence signal were followed for 24 hours, both the spectral changes and the degree of fluorescence polarization were recorded. No change in the shape of the PL spectra was observed during the self-organization process while significant decrease of the intensity was detected in time. The intensity change during time shows significantly different dependence on the concentration of the Somatostatine reflecting complex kinetics of the molecular association.

Acknowledgement

This work was supported by the French-Hungarian Intergovernmental S&T Cooperation Programme (Project No: FR-20-09).

References

[1] van Grondelle, W., López Iglesias, C., Coll, E., Artzner, F., Paternostre, M., Lacombe, F., Cardus, M., Martinez, G., Montes, M., Cherif-Cheikh, R., Valéry, C. *J. Struct. Biol.* **160** (2007) 211-223.

[2] Gazit, E., *FASEB J.* **16** (2002) 77–83.

[3] Makin, O.S., Atkins, E., Sikorski, P., Johansson, J., Serpell, L.C., *Proc. Natl. Acad. Sci. USA* **102** (2005) 315–320.

[4] Kunsági-Máté, S., Lecomte, S., Ortmann, E., Kunsági-Máté, É., Desbat, B., *J. Incl. Phenomena and Macrocycl. Chem.*, 66 (2010) 147-151.

P-14

SELECTIVE AMPEROMETRIC DETERMINATION OF PYROCATECHOL AND PHENOL IN WINES WITH FLOW-INJECTION ANALYSIS

András Kiss[1]*, Zsolt Bánfai[1], Karel Lacina[2], Jirí Zeravik[2], Petr Skládal[2], Géza Nagy[1]

(1) Department of General and Physical Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary, aldimeola3@gmail.com

(2) Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic

A selective amperometric flow-injection sensor for phenolic substances was constructed for determination of pyrocatechol and phenol in wine. Tyrosinase and laccase enzymes were combined with the four-channel screen-printed sensor, individual enzymes, their mixture and a reference layer were immobilized on the graphite-based working electrodes in the array. The enzymes are oxidizing phenols and thus obtained quinones become reduced on the working electrodes thus providing four values of current. The electrode covered with tyrosinase and laccase together was selective to pyrocatechol (Fig 1.), the selectivity coefficient was $k_{a,i} = 0.11$. The electrode modified with tyrosinase measured phenol and pyrocatechol together. Thus, the concentration of both substances can be calculated. It is supposed that the observed selectivity was due to production of the phenoxyl radical in the enzymatic reaction with laccase from phenol, but not from pyrocatechol. The radical was not able to reach the surface of the electrode as it was probably reacting with components inside the biolayer before reaching the electrode surface. The sensitivities of the sensor were 5.11 and 4.4 nA/μM for pyrocatechol and phenol, respectively. Surprisingly, the sensor was not sensitive to resorcinol and hydroquinone.

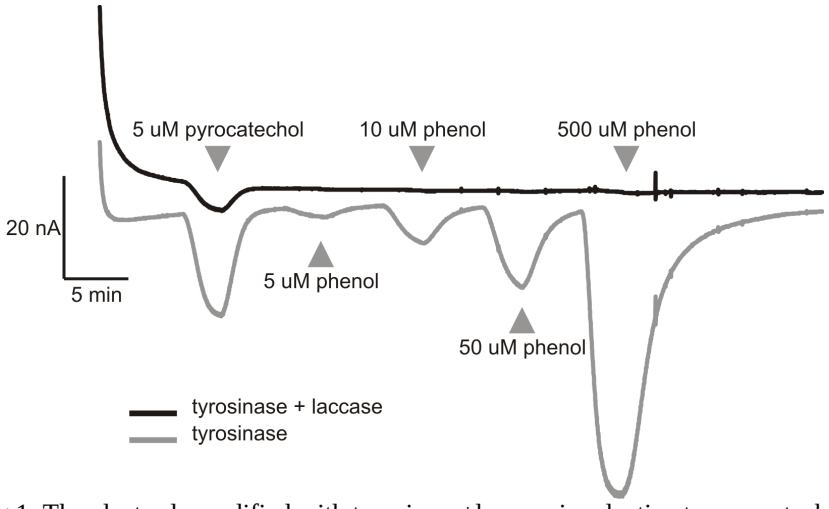


Fig 1. The electrode modified with tyrosinase+laccase is selective to pyrocatechol.

Acknowledgement

This research was supported by the Czech-Hungarian joint research project MEB 040914.

P-15

CO_2 PARTIAL PRESSURE IMAGING IN GAS PHASE WITH SCANNING ELECTROCHEMICAL MICROSCOPY (SECM)

András Kiss* and Géza Nagy

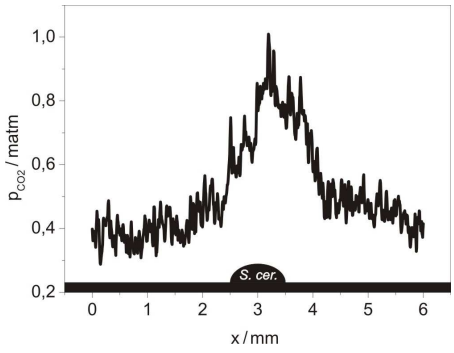
Department of General and Physical Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary, g-nagy@ttk.pte.hu, aldimeola3@gmail.com

Scanning electrochemical microscopy is a powerful technique for studying surfaces, surface reactions and concentration profiles. It is based on micro size selective electrochemical transducer, high precision positioning devices and computer controlled data collection, evaluation and image formation. In most of the SECM studies the measurement tip is scanned in liquid phase.

In our recent work a micro size Severinghaus-type carbon dioxide measuring cell was developed. The cell contains an antimony disc electrode of a few μm diameter and a silver-chloride coated silver reference electrode. Preparing the cell assembly a glass-coated antimony fiber and a silver wire (d = 0.1 mm) were cemented together with epoxy glue in a glass capillary. A drop of 10^{-2} M sodium-bicarbonate and 10^{-1} M sodium-chloride solution was put on the tip to create a thin sensing film.

The carbon dioxide in the surrounding gas phase interacting with the sensing film determines the pH in the sensing film. The antimony disc detects this giving potentiometric signal. Detailed investigation of the analytical properties of the microcell, like CO_2 partial pressure response, response time, stability, internal resistance, selectivity, interferences have been performed.

By applying the SECM cell as measuring tip, the local CO_2 concentration in gas phase could be detected. In this way CO_2 producing active microbial spots could be detected through air gap, without interaction of the tip by stirring or causing any other kind of invasion. CO_2 partial pressure – lateral distance, one dimensional SECM image can be seen in the figure below. It was recorded by scanning the tip in the gas phase about 500 μm over a colony of yeast grown on anutrient agar-agar medium. As it can be seen the CO_2 partial pressure could achieve 2.6 times higher value than it is in atmosphere (390 μatm)



pCO_2 profile above a yeast (*S. cerevisiae*) colony measured with a Severinghaus-type CO_2 sensor using SECM.

References

[1] Severinghaus, John W., Bradley, A. Freeman, *Appl. Physiol.* **13** (1958) 515-520.
[2] Beyenal, H., Davis, C. C., Lewandowski, Z., *Sens. and Act. B.* **97** (2004) 202-210.

P-16

DETERMINATION OF GASEOUS H_2S USING CARBON FIBER MICROWIRE ELECTRODE PAIR

László Kiss[1]* and Barna Kovács[1,2]

(1) DDKKK Inc., Pécs, Hungary,

(2) Department of General and Physical Chemistry, University of Pécs, Pécs, Hungary

kovacs1@gamma.ttk.pte.hu

The quantification of gaseous H_2S content in biogas and above biological systems is a crucial task, because of its toxicity. This gas is formed by different sources, such as compost, clamp or marshland. Also in food industry, its level is an informative factor of the food freshness, because the anaerobic biological activity results also in hydrogen sulphide.

Electrochemical gas sensors could be miniaturize easily; thus they have been gaining more and more attention because of the need for portable and easy-to-fabricate devices. Usually amperometric measurements are performed in solvents containing background electrolyte. Interestingly few paper in the literature report on the use of amperometric detection directly in air or in gaseous samples [1,2].

In this work bare and Cu_xS covered carbon fiber microelectrodes were used for the amperometric determination of H_2S in gas samples. Carbon fiber microelectrodes proved to be sensitive and selective in the determination of several biologically important compounds; recently efforts were made to use carbon material as electrode for the measuring the content of certain compounds in vapor. On the other hand, metal sulphides are excellent anode materials in H_2S fuel cell applications, therefore thin Cu_xS deposited carbon fiber microelectrodes were also tested as promising electrode materials.

The microelectrodes (30 μm in diameter) were glued parallel to each other on plexi glass by keeping a small, approximately 10 μm air gap between them. A portable potentiostat served as detection unit. The carbon fiber microelectrode pair (anode and cathode) was exposed to gaseous H_2S, and a current increase was obtained when using 2 V polarizing potential. Both the bare and the covered electrodes showed recoverable signals when changing the sample back to pure air. The cross sensitivity for water vapor and ammonia gas were also tested. The carbon electrodes were found to be highly selective for H_2S and fully reversible. In case of Cu_xS covered electrodes higher current intensity was measured in the presence of the analyte, while their selectivity was significant lower than that was measured at the bare carbon fibers.

References

[1] Fam, D.W.H., Tok, A.I.Y., Palaniappan, Al., Nopphawan, P., Lohani, Anup, Mhaisalkar, S.G., *Sensors and Actuators B: Chemical* **138** (2009) 189-192

[2] Wang, Y., Yan, H., Wang, E., *Sensors and Actuators B: Chemical* **87** (2002) 115-121

P-17

EFFECT OF SOLVATION SHELL COMPOSITION ON THE WEAK MOLECULAR INTERACTIONS OF AROMATIC MOIETIES

Sándor Kunsági-Máté[1]* and Koichi Iwata[2]

(1) Department of General and Physical Chemistry, Faculty of Sciences, University of Pécs, Ifjusag 6., H-7624 Pécs, Hungary. kunsagi@gamma.ttk.pte.hu

(2) Department of Chemistry, Faculty of Science, Gakushuin University, 1-5-1 Mejiro, Toshima-ku, Tokyo 171-8588 Japan.

The growth and stability of nanostructures at atomic scale are known to be highly affected by molecular environment. This property has significant consequences at wide scale of nanotechnology where the environment of the growth and also the environment of applications are a complex matrix. In our previous works examples were collected from very different field of applications: chemical sensors where the dynamic motions of solvent molecules affects the sensitivity and selectivity [1], evolution of the color of red wines where the anthocyanine-polyphenol interactions are affected by the ethanol-water ratio [2,3] or biological applications where the solvation shell formed in binary solutions highly affect the conformational transitions of proteins [4]. To get deeper insights into this property the solvation dynamics of solvent molecules around a family of phenolic derivatives was examined in ethanol – water mixtures. Result shows significant change of the solvent relaxation times at a typical molar fraction of ethanol (Figure 1). We have shown that the composition of the solvation shell in binary mixtures is far from the composition of the bulk solution [5] and this property was identified as background of the above effects at molecular level.

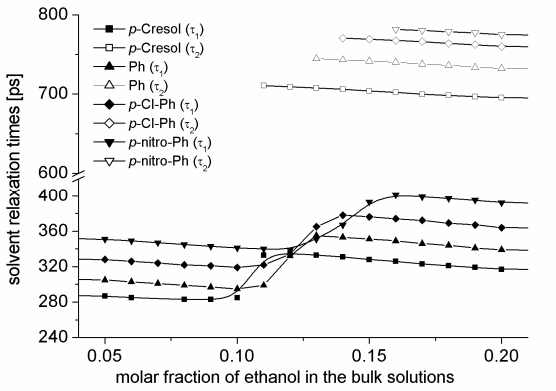


Figure 1 : Solvent relaxation times observed in binary water-ethanol solutions of phenol derivatives

Acknowledgement

This work was supported by the Japan-Hungarian Intergovernmental S&T Cooperation Programme (Project No: JP-14-09).

References

[1] Kunsági-Máté, S., Csók, Zs., Tuzi, A., Kollár, L., *J. Phys. Chem. B* 112 (2008) 11743-11749.

[2] Kunsági-Máté, S., Kumar, A., Sharma, P., Kollár, L., Nikfardjam, M.P., *J. Phys. Chem. B* 113 (2009) 7468-7473.

[3] Kunsági-Máté, S., Kollár, L., *Int. J. Chem. Modeling* 1(2) (2009) 177-192.

[4] Kunsági-Máté, S., Lecomte, S., Ortmann, E., Kunsági-Máté, É., Desbat, B., *J. Incl. Phenomena and Macrocycl. Chem.*, 66 (2010) 147-151.

[5] Kunsági-Máté, S., Iwata, K., *Chemical Physics Letters* 473 (2009) 284-287.

P-18

FOUR-CHANNEL ENZYME BIOSENSOR FOR DETERMINATION OF PHENOLIC COMPOUNDS IN WINE

Karel Lacina[1]*, András Kiss[2], Zsolt Bánfai[2], Jirí Žeravík[1], Petr Skládal[1] and Géza Nagy[2]

(1) Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic, lacinak@chemi.muni.cz
(2) Department of General and Physical Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary

The taste of wine is a complex characteristics influenced by the presence of different compounds [1,2]. For proper characterization of wine samples in addition to alcohol and sugars [2], phenolic compounds must be assigned. For this purpose, the 4-channel electrochemical biosensor was designed with sensitivity towards different phenolic substances. The following phenols-specific enzymes were utilized: tyrosinase (TYR), laccase (LAC) and horseradish peroxidase (HRP). The biosensor was realized by immobilization of enzymes on the surfaces of 4 individual working electrodes screen-printed on a common ceramic support. The biosensing layers consisted of the selected enzyme, albumin and glutaraldehyde. The sensing channels (no. 1 to 4) contained: 1. LAC, 2. HRP, 3. TYR, 4. BSA. Thus modified sensor was placed in the automated flow-through system consisting of peristaltic pump, home-made electrochemical cell and 6-channel switching valve supplying the system with appropriate buffer and samples. The multichannel detector ImmunoSMART and an own software LabTools realized recording of data and automation of the measuring procedure.

The function of the biosensor was based on the enzymatic oxidation of phenolic compounds to quinones which were reduced on the working electrode. This was directly accomplished in the case of LAC and TYR. For the HRP channel, H_2O_2 was present in the buffer solution. The obtained cathodic current was thus directly proportional to the concentration of particular phenols in a sample. The last electrode modified with inert protein served as a blank channel for proper discrimination of other electroactive interferences in wine. Different types of phenolic compounds were probed with the developed 4-channel biosensor to evaluate its selectivity. Finally, performance of the biosensor on real samples of wine was tested.

Acknowledgements
The research was supported by the Czech-Hungrian collaborative project no. MEB 040914 and by the National Program of Research II project no. 2B08035 (Ministry of Education of Czech Republic).

References
[1] Žeravík, J., Hlaváček, A., Lacina, K., Skládal, P., *Electroanalysis* **21** (2009) 2509.
[2] Lacina, K., Žeravík, J., Skládal, P., Biosensors 2010 congress, Glasgow, UK, poster 0715: Four-channel enzyme biosensor for determination of sugars in wine.

P-19

ANALYSIS OF INORGANIC ANIONS IN MINERAL WATERS WITH ISOTACHOPHORESIS

Nándor Lambert[1]* and Ferenc Kilár[1,2]

(1) Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary

(2) Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624 Pécs, Hungary

The cationic constituents of mineral waters are generally presented on the container, but less information is given about the anionic compounds, although, they can be harmful to health. The aim of our study was to quantify the chloride, sulphate, nitrate, nitrite, fluoride and phosphate ions in different mineral water samples and a tap water sample.

We used a column coupled isotachophoretic system [1] to identify the inorganic compounds in various mineral waters and a tap water from Hungary.

Each sample contained fluoride in different amounts. Nine out of ten samples comprise sulphate ion, and nitrite and phosphate ions were detected in three cases (including the tap water). The chloride content, found in three mineral water samples, was much higher than in the tap water.

In several cases the results showed significant differences between the measured and reference data [2].

This study shows that the use of the isotachophoretic system is suitable for the measurement of the anionic constituents of environmental samples, providing a reliable, simple and fast determination.

References

[1] Everarerts, F. M., Beckers, J. L., and Verheggen, Th. P. E. M. (1976) Isotachophoresis, theory, instrumentation and applications, Journal of Chromatography, Amsterdam, 13-20

[2] Zelensky, I., Zelenska, V., Kaniansky, D., Havasi, P., Lednárová, V., *J. Chromatogr*. **294** (1984) 317-327

P-20

SELF ASSOCIATION PROCESS OF SOMATOSTATIN

Sophie Lecomte[1], Wilmar van Grondelle[2], Céline Valéry[2], Katalin Kispál[1,3], Erzsébet Szász[3], Bernard Desbat[1] and Sándor Kunsági-Máté[3]

(1) CBMN, UMR 5248, CNRS-Université Bordeaux 1-ENITAB, IECB, Pessac, France s.lecomte@cbmn.u-bordeaux.f

(2) Ipsen Pharma, Sant Feliu de Llobregat, Barcelona, Spain.

(3) Department of General and Physical Chemistry, University of Pécs, Ifjusag 6., H-7624 Pécs, Hungary.

Natural Somatostatin-14 (Figure 1) is a small cyclic neuropeptide hormone that is unique in its broad inhibitory effects on endocrine secretions. It was not only shown to regulate growth hormone, glucagon, insulin and gastrin secretions, but also to modulate cognitive processes. For Somatostatin-14 at low concentrations in water, as well as in other solvents, earlier works showed a high propensity for antiparallel beta-sheet and beta-turn secondary structures. A model conformation in solution based on a beta-hairpin was proposed and further related to the Somatostatin biological activity [1,2]. 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 [3]. These non-covalent structures are built on antiparallel beta-sheet hydrogen-bond networks that are developed from the native Somatostatin beta-hairpin. We demonstrated that the Somatostatin aromatic residues 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. Photoluminescence and fluorescence polarization measurements were applied for following the association processes. Decrease of the PL signal highlighted the interactions of the aromatic moieties of the amino acids while the degree of polarization increased showing slower rotational motions of the associated species. The long-term time dependence reflects fibrillation process rather than the association of single Somatostatin molecules.

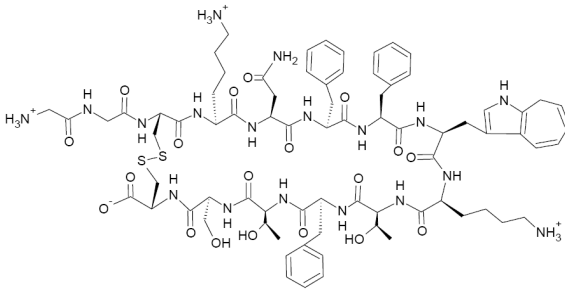


Figure 1 : Structure of Somastotatin-14

Acknowledgement

This work was supported by the French-Hungarian Intergovernmental S&T Cooperation Programme (Project No: FR-20-09).

References

[1] Holladay, L.A., Puett, D., *Proc. Natl. Acad. Sci. USA* **73** (1976) 1199–1202.

[2] Holladay, L.A., Rivier, J., Puett, D., *Biochemistry* **16** (1977) 4895–4900.

[3] van Grondelle, W., López Iglesias, C., Coll, E., Artzner, F., Paternostre, M., Lacombe, F., Cardus, M., Martinez, G., Montes, M., Cherif-Cheikh, R., Valéry, C., *J. Struct. Biol.* **160** (2007) 211-223.

P-21

MASS SPECTROMETRIC ANALYSIS OF MYCOLIC ACIDS AS MYCOBACTERIAL LIPID BIOMARKERS

Gábor Maász and László Márk*

Institute of Biochemistry and Medical Chemistry, University of Pecs, H-7624 Pecs, Szigeti str. 12, Hungary, laszlo.mark@aok.pte.hu

The cell wall of mycobacteria includes an unusual outer membrane of extremely low permeability. This cell envelope consists of a characteristic cell wall skeleton, a mycoloyl arabinogalactan peptidoglycan complex, and related hydrophobic components that contribute to the cell surface properties. In this study 1400-year-old as well as recent mycolic acids as unique tuberculosis biomarkers have been extracted and identified by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) and Fourier transform infrared spectroscopy (FT-IR). The data suggest that the MALDI TOF MS has potential as a rapid and reproducible technique for the detection and identification of ancient mycobacterial infections.

P-22

ENDOTOXINS WITH HIGHLY SENSITIVE MICROFLUIDIC CE ANALYSIS

Lilla Makszin[1*], Anikó Kilár[2,3], Péter Felső[1], Béla Kocsis[2] and Ferenc Kilár[1,3]

(1) Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624 Pécs, Hungary, lilla.makszin@aok.pte.hu

(2) Institute of Medical Microbiology and Immunology, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624 Pécs, Hungary, bela.kocsis@aok.pte.hu

(3) Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary, aniko.kilar@aok.pte.hu, ferenc.kilar@aok.pte.hu

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 pure and 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.

References

[1] Kilár, A., Farkas, V., Kovács, K., Kocsis, B., Kilár, F., *Electrophoresis* **29** (2008) 1713–1722.

[2] Kilár, A., Péterfi, Z., Csorba, E., Kilár, F., Kocsis, B., *J. Chromatogr. A* **1206** (2008) 21-25.

[3] Makszin, L., Kilár, A., Felső P., Kocsis, B., Kilár, F., *Electrophoresis* (2010) – submitted for publication

P-23

ELECTROCHEMICAL SENSOR DEVELOPMENT FOR MEASUREMENT OF ROS IN ETHANOL INDUCED STRESS

Lívia Nagy[1*], Tünde Angyal[1], Matsumoto Akiko[2], Jan Pribyl[3], Petr Skladal[3], Géza Nagy[1]

(1) *Institute of Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary, lnagy@ttk.pte.hu,*

(2) Department of Pharmaceutical Science, Univeristy of Colorado, Aurora, CO 80045, USA

(3) Department of Biochemistry, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic

The participation of reactive oxidizing species (ROS) in different physiological processes, including aging, signal transduction and some kind of immune functions is proved and their role is intensively investigated.

It is well known that metabolism of ethanol causes oxidative stress in liver tissue. Oxidative stress is generated through the various pathways related to ethanol metabolism (e.g., ALDH, microsomal ethanol oxidizing system), thus leading to hepatic disease. We have previously reported that one of the genetic polymorphisms affects oxidative stress caused by ethanol in model animals [1,2]. In those studies the experimental animals had to be sacrificed for the analysis. Obtaining closer view and saving life of experimental animals could be resulted by using a proper method for local monitoring of ROS species.

Therefore, we decided to introduce into our studies new effective and reliable methods that allow in vivo monitoring. Electrometric microsensors are often used monitoring different species in living tissues of anesthetized experimental animals. However, their analytical values, like selectivity, lower limit of detection needed enhancement. Recently selectivity improvements have been achieved by employing special, electrochemically prepared polymer layers [3]. In our work the preparation procedures, the stability, and selectivity of the size exclusion membranes applied on microelectrode surfaces have been investigated as well as their effect on lower limit of determination. In order to increase sensitivity of detection with coated amperometric electrodes, the method of periodically interrupted amperometry (PIA) has been introduced [4].

In this presentation we shortly introduce our recent results achieved working out an electrometric sensor and a method applicable for ROS measurements. Molecule modelling, in situ atomic force microscopy (AFM) and quartz crystal microbalance (QCM) experiments combined with controlled potential electrolysis [5] were employed in developing the selectivity providing polymer layer.

References

[1] Matsumoto, A., Kawamoto, T., Mutoh, F., Isse, T., Oyama, T., Kitagawa, K., Nakayama, K., Ichiba, M., *Pharmacogenetics & Genomics* **18** (2008) 847-852.

[2] Matsumoto, A., Ichiba, M., Horita, M., Yamashita, Z., Takahashi, T., Isse, T., Oyama, T., Kawamoto, T., Tomokuni, K., *Alcohol* **41** (2007) 57-59.

[3] Nagy, L., Nagy, G., Gyurcsányi, R. E., Lindner, E., Neuman, M. R., J. *Biochem. and Biophys. Meth.* **53** (2002) 165-175

[4] Nagy, L., Kálmán, N., Nagy, G., *J. Biochem. Biophys. Meth.* **69** (2006) 133

[5] Pribyl, J., Jilek, M., Skladal, P., 2010. *Nanoeasure2010* Krakow, Poland 3-4 June

P-24

AMPEROMETRIC CELL FOR ENZYME ACTIVITY MEASUREMENT

Lívia Nagy*, Zsolt Bánfai and Géza Nagy
Department of General and Physical Chemistry, Faculty of Sciences, University of Pécs, 7624 Pécs, Ifjúság u. 6. Hungary
lnagy@ttk.pte.hu

Small-volume electrochemical cell with three electrodes for enzyme activity measurements was prepared. It was a further developed variety of the one used before in this laboratory. [2]. Using the cell the enzyme-catalyzed reaction takes place in a thin film inside the cell, and the concentration changes are followed in time with an amperometric detector. The combination of the thin-layer reaction medium and surface detection provides high sensitivity with very low sample volumes.

Standard spectrophotometric enzyme activity measuring procedure was used for comparison. Tyrosinase enzyme EC 1.14.18.1 was the analyte in these studies. Learning that the amperometric flat form microcell provides a good and easy way for measuring enzyme activity in microliter volume samples it was used for analyzing different enzyme sources, like fruit juices and plant tissues, vegetable pulps or slices.

Using different separation technique like centrifuging, sedimentation, optically observed manual selection the high activity fractions were isolated for amperometric biosensor preparation.

For measuring low polyphenol oxidase activity of natural enzyme sources the recently developed periodically interrupted amperometry (PIA) [2,3] was used for obtaining higher amperometric current response. In these works dopamine, was employed as substrate. The detection is based on electrochemical reduction of the quinine formed in the enzyme catalyzed reaction of oxygen and phenolic compounds.

After analyzing several natural sample sources (apples, bananas, potato) and their fractions the highest activity of polyphenol oxidase was found in certain banana pulp fraction.

In our presentation the measuring cell and the procedure worked out will be introduced and the obtained enzyme activity data obtained will be discussed.

References

[1] Nagy, G., Xu, C.X., Buck, R.P,. *Anal. Chem*. **70** (1998) 2156-2162.
[2] Nagy, L., Kálmán, N., Nagy, G., *J. Biochem. Biophys. Methods* **69** (2006) 133–141.
[3] Takátsy, A., Csóka, B., Nagy, L., Nagy, G., *Talanta* **69** (2006) 281–285.
[4] Lee, W.C., Yusof, S., Hamid, N.S.A., Baharin, B.S., *Journal of Food Engineering* **75** (2006) 473-479.

P-25

HEAVY METAL ANALYSIS IN ASH OF BIOMASS

Lívia Nagy*, Szabina Geges and Géza Nagy
Department of General and Physical Chemistry, Faculty of Sciences, University of Pécs, 7624 Pécs, Ifjúság u. 6. Hungary
lnagy@ttk.pte.hu

The fossil fuel burning technologies provide the ovelwhelming part of the energy needed today. In trying to increase the ratio of renewable energy sources biomass like wood appears as a straight forward choice.

In the last 10 years, the wood heating, as well as energy production enjoyed a growing popularity owning to its carbon-dioxide-neutrality and renewable nature. Increased use of wood heating produces growing amount of ash year by year.

The wood ash contains valuable plant nutrients, though it is alkaline. Actually it could be used as fertilizer in remediation of acidic soils of forests or farmlands. However, various studies proved that wood ashes from different plants gown in different locations contain high heavy metal concentrations [1, 2]. This does not allow usage of the wood ash as fertilizer. Austrian and Norwegian researchers suggest that the ash has to be checked the same way as the sewage treatment dirt before deposition or using as fertilizer [3].

There are highly sensitive electrochemical methods for analysis of concentration of heavy metals. Comparing them with other analytical methods they are simpler and more cost efficient. They can be applied for analysis of heavy metals in wood ashes.

In our work the methods of electrometric heavy metal analysis were investigated carrying out measurements in ash samples. Different working electrodes, different sampling procedures, different accumulation and measuring methods were compared.

In our work inverse voltammetry with the square wave detection method was showed the best performance applied. In case of application of screen printed carbon nanotube based electrode, lower limit of detection as low as 10 ng/ kg could be reached, due to the enrichment step of the method. The accuracy of the measurements was checked with measurements of the same samples by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Measurements of acid extracts of ash samples generated by burning energy grass and energy grass-wood co-combustion of were also carried out.

In our presentation the sample pretreatment procedure and the measuring method used will be described in details.

References

[1] Omil, B., Piñeiro, V., Merino, A., *Science of the Total Environment* **381** (2007), 157 - 168.

[2] Ribbing, C., *Waste Management* **27** (2007) 1428 - 1435.

[3] Reimann, C., Ottesen, R.T., Andersson, M., Arnoldussen, A., Koller, F., Englmaier, P., *Sience of the Total Enviroment* **393** (2008) 191 - 197.

P-26

EFFECT OF THE pH OF THE ANOLYTE AND CATHOLYTE SOLUTIONS IN CIEF SEPARATION WITH SEQUENTIAL INJECTION SETUP

Csilla Páger[1*], Andrea Vargová[2] and Ferenc Kilár[1,2]

(1) Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12, H-7624 Pécs, Hungary

(2) Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary

Isoelectric focusing in fused silica capillary (cIEF) is convenient for the characterization of samples that contain proteins or peptides, such as body fluids or proteomic samples. The analytes are separated according to their isoelectric points (p*I*), in a pH gradient developed by ampholytes upon applying an electric field. One advantage of cIEF is the concentration of the analytes in the pH gradient because each component migrates to a specific position where the pH is equal to its p*I*. In the common cIEF the analytes and the carrier ampholytes are injected in a mixture. The sandwich injection set-up (in which the analysed sample compounds are injected between two ampholyte zones) offers an effective separation of amphoteric compounds having p*I* values outside the pH range of the applied ampholyte. During the separation, the evolving pH gradient is affected by various experimental conditions, such as the type of the ampholyte solutions, the lengths of the ampholyte zones, and the applied voltage. Our aim was to examine the effect of the pH of the anolyte and catholyte solutions on the resolution of the separation. Ampholytes with narrow pH range (covering pH range 7-9) were applied in uncoated capillary and aminomethylated nitrophenol dyes (with p*I*s 5.3, 6.4, 6.6, 7.9 and 10.4) were used as p*I* marker samples. Changing the pH of the anolyte between 2.5 and 7.5 and altering the pH of the catholyte between 11 and 8, may cause that analytes having p*I*s outside the pH range of the applied ampholyte migrate out from the zone of the ampholytes (from the pH gradient). In this way the detection of the analytes can be improved by suppressing the presence of the ampholyte components.

Acknowledgements

The work was supported by the grants GVOP-3.2.1-0168, OTKA-K75717 and OTKA-NKTH-NI-68863.

P-27

THE ADSORPTION BEHAVIORS OF ANILINE ON CARBON NANOTUBES

Beáta Peles-Lemli[1,2*], Walter M.F. Fabian[2], László Kollár[3] and Sándor Kunsági-Máté[1]

(1) Department of General and Physical Chemistry,Faculty of Sciences,University of Pécs, Ifjusag 6., H-7624 Pécs, Hungary

(2) Institute of Chemistry, Karl-Franzens University of Graz, Heinrichstr. 28, A-8010 Graz, Austria

(3) Department of Inorganic Chemistry, Faculty of Sciences, University of Pécs, Ifjusag 6., H-7624 Pécs, Hungary

blemli@gamma.ttke.pte.hu

Because of their large surface area and high reactivity, carbon nanotubes (CNTs) are attractive materials as effective adsorbents for organic or inorganic chemicals [1]. Nowadays the adsorptions of the environmental contaminant aniline on CNTs have increasing attention [2,3] although the related mechanism have not clarified in detail. For better understanding the adsorption of this aromatic molecule on carbon nanotubes, in this work the molecular interaction between aniline and CNTs have been investigated. The fluorometric measurements highlighted the complexivity of the analyzed mechasims. The results [4] support the weak π-π interaction between the aromatic moeties of the nanotube and the aniline, rather than a charge transfer reaction suggested earlier. Furthermore, the calculated thermodynamic parameters indicated ratherthe pereference of physisorption than chemisorption as the dominant effect during the related processes [5]. These experimental results are in good agreement with the DFT calculations, which also indicating that the non-covalent π-π interaction plays the dominant role during the adsorption process of aniline onto the nanotube surface [6]. The results above might serve to improve the development of more efficient adsorbents or biosensors suitable for environmental applications.

Acknowledgments

The financial support of the Austrian-Hungarian Intergovernmental S&T Programme (AT-23/08) is highly appreciated. Parts of the calculations are performed on SunFire 15000 supercomputer located in the Supercomputer Center of the Hungarian National Infrastructure Development Program Office.

References

[1] Long, R.Q., Yang, R.T., *J. Am. Chem. Soc.* **123** (2001) 2058-2059.
[2] Xie, X., Gao, L., Sun, J. *Colloids Surf. A* **308** (2007) 54–59.
[3] Yang, K., Wu, W., Jing, Q., Zhu, L. *Environ. Sci. Technol.* **42** (2008) 7931-7936.
[4] Peles-Lemli, B., Ács, P., Kollár, L., Kunsági-Máté, S. *Fuller. Nano. Car. N.* **16** (2008) 247-257.
[5] Peles-Lemli, B., Kollár, L., Kunsági-Máté, S. *Fuller. Nano. Car. N.* **18** (2010) 234-242.
[6] Peles-Lemli, B., Matisz, G., Kelterer, A-M., Fabian, W.M.F., Kunsági-Máté, S. *J.Phys. Chem. C* **114** (2010) 5898-5905.

P-28

ADVANCES OF COMPUTER MODELING IN CAPILLARY ISOELECTRIC FOCUSING

Anna Takácsi-Nagy[1], Csilla Páger[1], Ferenc Kilár[1,2], Wolfgang Thormann[3]

(1) Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12, H-7624 Pécs, Hungary, atakacsi@gamma.ttk.pte.hu

(2) Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary, ferenc.kilar@aok.pte.hu

(3) Department of Clinical Pharmacology and Visceral Research, University of Bern, Murtenstrasse 35, 3010 Bern, Switzerland wolfgang.thormann@ikp.unibe.ch

Capillary isoelectric focusing (cIEF) is a high-resolution technique for protein separation based on differences in isoelectric points (p*I*). The samples are migrating in a stable pH gradient until they reach the pH equal to their isoelectric points, at which point the net charge and mobility are zero. In contrast to gel-based IEF, cIEF is rapid, easily automated and offers higher resolution and quantitation capabilities. The method has been long used to separate, isolate, purify and analyze a variety of proteins.

In our Institute we developed a consecutive "sandwich" injection, in which ampholyte–sample–ampholyte components are plug in the capillary for analysis. This new set-up allows the separation of amphoteric compounds having p*I* values outside the pH region of the ampholytes applied in the capillary with high precision [1]. In practical experiments we worked with substituted aminomethyl-phenol dyes (p*I*: 5.3, 6.4, 6.6, 7.9, 10.4) and ampholytes with pH range 6-8 and 7-9 using sequential injection of ampholyte and sample zones.

Our goal was to confirm the mechanism of the consecutive injection with computer modeling. The results of the simulations show that the new injection protocol can be used to separate the sample components having p*I* values outside of the pH range of the ampholytes.

Reference

[1] Kilár, F., Végvári, Á., Mód, A., *J. Chromatogr. A* **813** (1998) 349-360

P-29

COMPARISON OF A NEWLY DEVELOPED FULLERENE(C60)-SILICA AND THE CONVENIENT SOLID PHASE EXTRACTION (SPE) MATERIALS FOR NONGLYCATED AND GLYCATED PEPTIDES

Anikó Takátsy[1]*, Katalin Böddi[1]*, Lajos Markó[2], István Wittmann[2], Róbert Ohmacht[1], Günther K. Bonn[3] and Zoltán Szabó[1]

(1) Department of Biochemistry and Medical Chemistry, Faculty of Medicine University of Pécs, Szigeti str. 12, 7624 Pécs, Hungary, aniko.takatsy@aok.pte.hu

(2) 2nd Department of Medicine and Nephrological Center, University of Pécs, Pacsirta str. 1, 7624 Pécs, Hungary

(3) Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University, Innrain 52a, 6020 Innsbruck, Austria

SPE plays a crucial role in bioanalytical research. In the present work a novel fullerene (C60)-derivatised silica material is compared with octadecyl (C18) – and triaconthyl (C30)-silicas regarding recoveries of peptides and sequence coverage of unmodified and glycated HSA and fibrinogen digests. C30- and C60(30) (C60-material prepared from a silica gel with a pore size of 30 nm)-SPE materials were found to be the two most prominent SPE materials. C60(30) demonstrated an outstanding value of sequence coverage and at low peptide concentrations it gave the best recovery values, however, in saturation C30-silica had higher binding capacity.

After nonenzymatic glycation the digests of fibrinogen and HSA were also separated what made the detection of considerably higher number of glycated peptides possible compared to the unfractionated digests. For HSA, ten new sites of glycation at lysine and arginine residues were explored. Using the detailed SPE/offline MALDI method the glycation sites on fibrinogen are first described by the authors. In the SPE experiments of glycated proteins C30-silica was found to be a competitive candidate compared to C60(30) regarding the number of glycated sites and the number of bound peptides as well. The selectivity of these materials differed; C30-silica adsorbed larger peptides, as long as C60(30) possessed excellent binding ability toward hydrophilic, arginine rich small peptides. According to the findings the C60(30) SPE-off-line MALDI method can be used for the investigation of polar constituents of complex biological samples.

Reference

[1] Böddi, K., Takátsy, A., Szabó, Sz., Markó, L., Márk, L., Wittmann, I., Ohmacht, R., Montskó, G., Vallant, R. M., Ringer, T., Bakry, R., Huck, C. W., Bonn, G. K., Szabó, Z., *J. Sep. Sci.* **32** (2009) 295-308.

P-30

EFFECT OF SURFACE MODIFICATION OF WASTE YEAST FROM BREWERY ONTO CADMIUM ADSORPTION

Katalin Tálos[1]*, Edina Ács[1], Alžbeta Hegedűsova[2] and Tímea Pernyeszi[1]

(1) Department of Analytical and Environmetal Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary, taloskatalin@gmail.com, ptimea@ttk.pte.hu

(2) Constantine the Philosopher University, Department of Chemistry, Faculty of Natural Sciences, Tr. A. Hlinku 1, SK-949 01, Nitra, Slovakia

The intensification of industrial activity during recent years is greatly contributing to the increase of heavy metals in the environment, mainly in the aquatic systems. The heavy metals pose serious health hazards through entry into the food chain by anthropogenic pathways. Biosorption, using biomaterials such as bacteria, fungi, yeast and algae, is regarded as a cost-effective biotechnology for treatment of high volume and low concentration complex wastewaters containing heavy metal(s) in order of 1 to 100 mg/L.

Biomass cell walls, consisting mainly of polysaccharides, proteins and lipids offer many functional groups which can bind metal ions such as carboxylate, hydroxyl, sulphate, phosphate and amino groups. In addition to these functional binding groups, polysaccharydes often have ion exchange properties. Pretreatment and killing of biomass either by physical or chemical treatments or crosslinking are known to improve the biosorption capacity of biomass.

Among the microorganisms used for biosorption, *Saccharomyces cerevisiae* is an inexpensive, readily available source of biomass for heavy metal removal from wastewater. In this study the waste *Saccharomyces cerevisiae* biomass was used as biosorbent for removal of large amounts of cadmium ion in aqueous solution. Waste yeast from brewery is available and its recycling can be solved by using as biosorbent material.

Our aim was to test and compare treated (by physical and chemical treatments) and untreated yeast cells for their capacity to adsorb cadmium ion. The effect of ethanol, heat and caustic treatments of biomass cells on cadmium biosorption was investigated. The experimental parameters were the followings: initial pH, adsorption time, initial cadmium concentration. The equilibrium and kinetic study of cadmium (II) sorption on treated and untreated biomass cells in aqueous suspension were analysed in all treated *Saccharomyces cerevisiae* biomass using batch technique.

Reference

[1] Göksungur, Y., Üren, S., Güvenç, U., *Bioresource Technology* **96** (2005), 103-109.

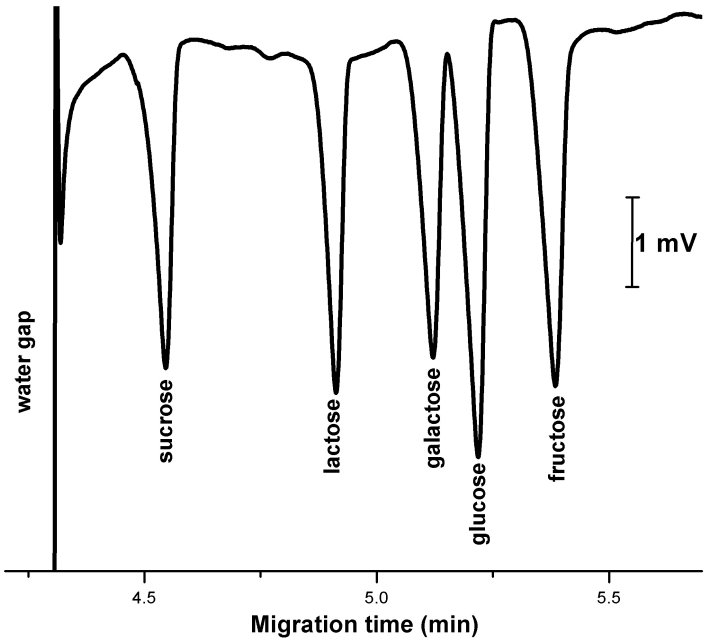
P-31

DETERMINATION OF NEUTRAL CARDOHYDRATES BY CAPILLARY ELECTROPHORESIS WITH CONTACTLESS CONDUCTIVITY DETECTION

Petr Tůma* and Eva Samcová

Institute of Biochemistry, Cell and Molecular Biology, Third Faculty of Medicine, Charles University in Prague, Ruská 87, 100 00 Prague 10, Czech Republic, petr.tuma@lf3.cuni.cz

A new capillary electrophoretic method for determination of neutral monosaccharides and disaccharides was developed [1]. The CZE separation was performed in very simple background electrolyte with composition 75 mM NaOH, pH 12.6. Under these conditions neutral carbohydrates are particularly dissociated and migrated as anions in electric field. Capacitively coupled contactless conductivity detection (C^4D) [2] was used for detection of non-absorbing carbohydrates with limit of detection on micromolar level. The determination of basic set of carbohydrates (glucose, galactose, fructose, sucrose and lactose) was accomplished in 5.5 min without need of sample derivatization. This method was successfully used for analysis of carbohydrates in orange and apple juice, milk, honey, coke and nutrient solution.



CE/C^4D separation of model mixture of mono- and disaccharides at concentration 100 mg/L in 50 % acetonitrile. Experimental conditions: background electrolyte, 75 mM NaOH (pH 12.6); capillary, total length 50 cm, 35 cm to C^4D, 10 µm id, 360 µm od; hydrodynamic injection at pressure 50 mbar for 100 s; separation voltage/current, +15 kV/+2.9 µA.

References

[1] Honda, S., *J. Chromatogr. A* **720** (1996) 337–351.

[2] Kubáň, P., Hauser, P.C., *Electrophoresis* **30** (2009) 176-188.

P-32

SECM STUDY OF CORROSION MEASUREMENT OF Zn^{2+} WITH ION SELECTIVE MICROELECTRODES

Ágnes Varga[1]*, Javier Izquierdo[2], Livia Nagy[1], Ricardo M. Souto[2], Géza Nagy[1]

(1) Department of General and Physical Chemistry, Faculty of Sciences, University of Pécs, 7624 Pécs, Ifjúság útja 6. Hungary

(2) University of La Laguna, Depto. Química Física, 38200 La Laguna (Tenerife), Spain

The fabrication of a new, solid contact, micropipette zinc ion-selective electrode suitable for use as potentiometric tip in scanning electrochemical microscopy (SECM) will be introduced. Owing to the low resistance, and long life times provided by the novel design, these electrodes can advantageously be used in corrosion studies, where the detection of local concentration of zinc ions evolving in surface reactions can give important information about the nature of the corrosion protecting process. Data showing the main analytical properties of the Zn^{2+} ion selective microelectrodes, like lifetime, resistance and ion activity response, will be shown. Preliminary findings using them in SECM imaging of zinc ion concentration profiles over corroding samples will be also given. The improvement of scanning rate achieved by lowering the tip resistance will be demonstrated. The small size giving high spatial resolution and the low resistance allowing faster scanning rate in SECM are major advantages of this new potentiometric SECM measuring tip.

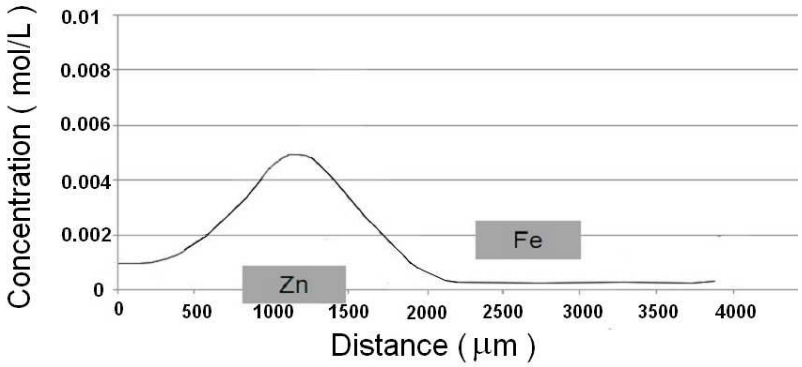


Figure of line scan displaying the variation of the zinc electrode potential measured at the ISE tip when traveling above an Fe-Zn galvanic couple immersed in 0.1 M KCl

References

[1] Gyetvai, G., Sundblom, S., Nagy, L., Ivaska, A., Nagy, G., *Electroanalysis* **19** (2007) 1116 -1122.

[2] Gyetvai, G., Nagy, L., Ivaska, A., Hernadi, I., Nagy, G., *Electroanalysis* **21** (2009) 1970-1976.

[3] Csóka, B., Kovács, B., Nagy, G., *Biosensors and Bioelectronics* **18** (2003) 141-149.

[4] Varga, Á., Izquierdo, J., Nagy, L., Bitter, I., Souto, R.M., Nagy, G.: Development of solid contact micropipette Zn ion selective electrode for corrosion studies (submitted)

P-33

RELEVANCE OF HUMIC ACIDS IN FOOD PRODUCTION, PROPHYLAXIS AND TREATMENT

Ladislav Vaško* and Janka Vašková

Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, P.J. Šafárik University, Tr. SNP 1, 040 66 Košice, Slovak Republic, ladislav.vasko1@upjs.sk

Humic acids (HA) are naturally occurring biological and chemical decomposed organic constituents that are formed from decayed plant matter by soil bacteria. Main natural sources of HA occur in silts, sapropelites, soil, humus, plat (10-40%), lignite and brown coal (10-30%). The highest content of HA in their oxydized products, oxyhumolites, could be observed (40-80%). They represent high-molecular polymeric aromatic substances characterised by complicated structure of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains, carbonyl, quinone but also semiquinone groups with sacharide and peptide constituents and significant physicochemical properties. Polyanionic character allows them to bind ions by physical or chemical mechanisms. Additionally, HA are described as strong chellating agens, with high adsorbing capacity, ion change molecules and buffers. Due to all properties HA are used in plant and animal production, but also in prophylaxis, prevention and either as therapeutical drugs in veterinary and human practices.

From plant production, enhancement in plant biomass rich in nutrients with lowered content of toxic components as heavy metals, PCB, dioxins, pesticides could be concluded. Entire positive effects from animal feeding trials are known [1]. They are used as antidiarrheal, antiinflammatory, immunostimulatory and antimicrobial agent but also as a part of the replacement therapy. Applied concentration range support gastrointestinal optimal pH, increased feed conversion ratio, egg yield, increase daily gain in weight, improved malnutrition, diarrhea, suppressed pathogenic and conditionaly pathogenic bacterial growth, positively affected growth of advisable microflora including probiotics [2] and what is important decreased overall morbidity and mortality.

HA application showed enhancement of quantity and currently quality in plant and animal food production. They, perspectively, could play an important role to obtain higher amount of healthy supplies. Except of healthy food production thay have an impotent role in putting up the health status of animals, humans and treatment mainly of gastrointestinal diseases and intoxication.

Acknowledgement

The study was supported by VEGA grant 1/0799/09.

References

[1] Vaško, L., Szanyi, G., Jeníková, J., *Slov. Vet. Čas.* **33** (3) (2008) 179-183

[2] Demeterová, M., Mariščáková, R., Pistl, J., Naď, P., Šamudovská, A., *Berl. Munch. Tierarztl. Wochenschr.*, **122** (9/10) (2009) 370-377

P-34

ACTIVATION OF ANTIOXIDANT DEFENCE BY SELECTED DIMETHYLAMINO CHALCONES IN MITOCHONDRIA

Janka Vašková[1]*, Ladislav Vaško[1], Juraj Guzy[1] and Pál Perjési[2]

(1) Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, P.J. Šafárik University, Tr. SNP 1, 040 66 Košice, Slovak Republic, janka.vaskova@upjs.sk

(2) Institute of Pharmaceutical Chemistry, Faculty of Medicine, University of Pécs, 7624 Pécs, Hungary

Open chain polyphenolic natural chalcones and their synthetic analogues, have been shown to possess a wide range of biological activities in a variety of experimental systems. Results of previous studies demonstrated that selected methyl-, methoxy- and hydroxyl- substituted compounds are able to modify antioxidant state of the mitochondria [1, 2]. As the mitochondrion is the main metabolic site with continuos generation of reactive oxygen species, modification of its function may contribute to cytotoxic activity of the compounds.

Activities of primary antioxidant defense enzymes superoxid dismutase, glutathione peroxidase, glutathione reductase and levels of reduced glutathione (GSH) on isolated rat liver mitochondria affected by four 4-dimethylamino substituted chalcone derivatives with different ring size were investigated.

E-2-(4´-dimethylamino-benzylidene)-1-tetralone pronounced antioxidant effect or was sufficient to compensate GSH substrate levels. The opposite effects by the *E*-2-(4´-dimethylamino-benzylidene)-1-benzosuberone (-indanone) were observed. There was no evidence of change in superoxid dismutase activities reflecting no superoxide generation. Although, glutathione related enzymes showed increased activities and reduced glutathione depletion allowed to consider peroxide formation.

Acknowledgements

This study was supported by VEGA grants 1/0624/08 and 1/0799/09 from the Slovak Grant agency for Science.

References

[1] Guzy, J., Vašková-Kubálková, J., Rozmer, Zs., Fodor, K., Mareková, M., Poškrobová, M., Perjési, P., *FEBS Lett.* **584** (2009) 567-570.

[2] Perjési, P., Kubálková, J., Chovanová, Z., Mareková, M., Rozmer, Zs., Fodor, K., Chavková, Z., et al., *Pharmazie* **63** (2008) 899-903.

Notes