9th INTERNATIONAL SYMPOSIUM

ON

INSTRUMENTAL ANALYSIS

FINAL PROGRAM

AND

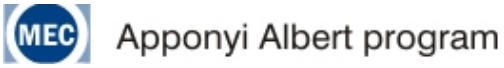
ABSTRACTS

June 29 – July 2, 2008

PÉCS

HUNGARY

SPONSORS



PÉCSI TUDOMÁNYEGYETEM

MAYOR OF PÉCS

Able&E-Jasco Magyarország Kft.
Alisca Coop
Aqua-Terra Lab Kft.
Edison House Kft.
Friesland Food
Kovács Cukrászda (Pécs)
Kromat Kft.
Magyar Kémikusok Egyesülete
Merck Kft.
PannonPharma Gyógyszergyártó Kft.
Pécsi Pékinas Kft
Richter Gedeon Nyrt.
Simkon Kereskedelmi és Szolgáltató Kft.
Spektrum-3D
Szentkirályi Ásványvíz
Zsolnay Porcelánmanufaktúra Zrt.

This symposium is the ninth in the series on Instrumental Analysis within the frame of the partnership between the cities of Pécs and Graz. The symposia were held in Pécs and in Graz alternatively since 1991 every second year. The scope of this symposium series is to give an overview of the activities in the field of instrumental analysis at the Universities of both cities.

It is an honour for the 9th Symposium on Instrumental Analysis to give opportunity for the celebration of

PROFESSOR GERALD GÜBITZ

KARL-FRANZENS-UNIVERSITY OF GRAZ

on the occasion of his 65th birthday.

Professor Gerald Gübitz is one of the scientists, who founded this series together with members of the Universities of Pécs and Graz. Since 1991, his lectures and those of his coworkers and students were always at the highest level during the long history of the symposia.
The past and present participants are grateful for his founding step and for the organization of many of these Symposia.

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

Organizing Committee

Ferenc Kilár (Chairman) (University of Pécs)
Martin G. Schmid (Co-Chairman) (Karl-Franzens-Univ. Graz)
Gerald Gübitz (Karl-Franzens University, Graz)
Attila Felinger (University of Pécs)
László Kollár (University of Pécs)
Walter Kosmus (Karl-Franzens-University Graz)
Sándor Kunsági-Máté (University of Pécs)
Ernst Lankmayr (Technical University Graz)
Géza Nagy (University of Pécs)
Reinhold Wintersteiger (Karl-Franzens-University Graz)

Scientific Committee

Attila Felinger (University of Pécs)
Gerald Gübitz (Karl-Franzens University, Graz)
Ernst Lankmayr (Technical University Graz)
Ferenc Kilár (Chairman) (University of Pécs)
László Kollár (University of Pécs)
Walter Kosmus (Karl-Franzens-University Graz)
Géza Nagy (University of Pécs
Martin G. Schmid (Karl-Franzens-University Graz)
Reinhold Wintersteiger (Karl-Franzens-University Graz)

Registration desk

Opening hours: June 29, 2008: 17:00-19:00 (Hotel Hunyor)
June 30, 2008: 8:00-12:00 (Faculty of Natural Sciences)

Location

The symposium will be held at the
Faculty of Natural Sciences, University of Pécs,
Pécs, Hungary
Ifjúság útja 6, H-7624, Pécs

Language: English

Poster session

Posters should be mounted by 9:00 a.m., June 30, and dismounted by 14:00 p.m., July 1, 2008. Authors are requested to be present at their posters during the poster session.

Social program

Lunch is organized at the Café Paulus (Ifjúság útja 6.) for the registered participants. The payment of the registration fee should be confirmed at the Registration Desk.

Accomodation

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.).

Excursion

An excursion is organized for the registered participants to Mohács and Villány on Tuesday, 1st of July, 2008. The buses will start at 14:00 from the main parking area of the Faculty of Medicine (Szigeti út 12.)

PROGRAM

June 29, 2008

17:00 – 19:00 Registration
Hotel Hunyor, Pécs, Jurisics Miklós utca 16.

19:00 Welcome reception
Hotel Hunyor, Pécs, Jurisics Miklós utca 16.

June 30, 2008

8:00 – 12:00 Registration
Faculty of Natural Sciences, Entrance Hall to the Conference Hall, Pécs, Ifjúság útja 6.

09:00 Opening of the Symposium
Faculty of Natural Sciences, Conference Hall, Pécs, Ifjúság útja 6.

Chairmen: Ferenc Kilár, Martin G. Schmid

Keynote Lecture

09:20 L-01 *Tamás Janáky, Zoltán Szabó, Szeliné Judit Szomor, Attila Csorba, Éva Szegő, Katalin Kékesi, Gábor Juhász, György Lévay*
University of Szeged, Szeged, Hungary
Molecular background of psychiatric diseases: proteomic studies

Lectures

10:00 L-02 *Gudrun Rieger, Franz Bucar*
Karl-Franzens University of Graz, Graz, Austria
Variation of flavonoid profiles in medicinal plants collected at different altitudes

10:20 L-03 *Attila Gáspár, István Bácsi, Menake E. Piyasena, Frank A. Gomez*
University of Debrecen, Debrecen, Hungary
Simple fabrication of fritless chromatographic microchips

10:40 Coffee break

Chairmen: Franz Bucar, Attila Felinger

Keynote Lecture

11:00 L-04 *Viktor Farkas, Melinda Rezeli, Ákos Végvári, Ferenc Kilár, Stellan Hjertén*
University of Uppsala, Uppsala, Sweden
Numerical calculations of the reversible and irreversible interaction of an analyte to the wall of the separation channel in CE and microchip electrophoresis. Are these interactions the cause of the low reproducibility of CE-MS experiments with proteins?

Lectures

11:40 L-05 *Margit Winkler, Norbert Klempier*
Technical University of Graz, Graz, Austria
Enantioseparation of N-protected β-and γ-amino acids for biocatalytic nitrile hydrolysis

12:00 L-15 *Attila Felinger, Péter Vajda, Szymon Bocian, Bogusław Buszewski*
University of Pécs, Pécs, Hungary
Characterization of stationary phases for reversed-phase liquid chromatography by solvent and solute adsorption phenomena

12:20 L-07 *Doris Kühnelt, Dijana Juresa, Norbert Kienzl, Kevin A. Francesconi*
Karl-Franzens University of Graz, Graz, Austria
The application of HPLC-mass spectrometry for the elucidation of selenium metabolism in mammals

12:40 L-08 *Anikó Kilár, Zoltán Péterfi, Eszter Csorba, Zoltán Szabó, Ferenc Kilár, Béla Kocsis*
University of Pécs, Pécs, Hungary
Capillary electrophoresis chips for screening of endotoxin chemotypes from whole-cell lysates

13:00 Lunch

14:00-16:00 **Poster discussion**

15:00 **Commercial presentations**

Chairmen: Tamás Janáky, Michael Murkovic

Keynote Lecture

16:00 L-09 *Andreas Kungl*
Karl-Franzens University of Graz, Graz, Austria
Proteoglycanomics: the sugar side of drug development

Lectures

16:40 L-10 *Christine Grabner, Rudolf Bauer, Wolfgang Schühly*
Karl-Franzens University of Graz, Graz, Austria
Separation of biphenyl-type lignans from Magnolia grandiflora by using Fast Centrifugal Partitioning Chromatography (FCPC)

17:00 L-11 *Dominique Cavagnat, Joelle Mascetti, Daniel Blaudez, Sabine Castano, Bernard Desbat*
Université Bordeaux 1, Pessac, France
Study of the molecular organization at the air/water interface using PMIRRAS, Surface pressure and BAM methods

17:20 L-12 *Victoria Samanidou, E. Evaggelopoulou, Martin Trötzmüller, Xinghua Guo, Ernst P. Lankmayr*
University of Thessaloniki, Thessaloniki, Greece
Multiresidue determination of seven quinolones antibiotics in gilthead seabream using liquid chromatography-tandem mass spectrometry

17:40 L-13 *Tünde Kupi, Pál Gróf, Miklós Nyitrai, József Belágyi*
University of Pécs, Pécs, Hungary
Interaction of formin with spin-labeled actin

19:00 Reception by the Mayor of Pécs (Faculty of Medicine, Szigeti út 12.)

July 1, 2008

Chairmen: Wolfgang Schühly, Miklós Kellermayer

Keynote Lecture

09:00 L-14 *Frank M. Sinner, Christina Gatschelhofer, Agnes Mautner, Franz Reiter, Michael R. Buchmeiser, Andreas Zimmer, Karin Wernig, Thomas R. Pieber*
Joanneum Research Forschungsgesellschaft mbH, Graz, Austria
Monolithic capillary columns prepared by ring-opening metathesis polymerization and their application in medical research

Lectures

09:40 L-06 *László Kollár, László Jánosi, Tamás Kégl*
University of Pécs, Pécs, Hungary
NMR investigations of the platinum-alkyl/aryl - B(C6F5)3 'in situ' systems as catalysts

10:00 L-16 *Bernd Trathnigg*
Karl-Franzens University of Graz, Graz, Austria
Prediction of retention in liquid chromatography of polymers

10:20 L-17 *Zsolt Mártonfalvi, Pasquale Bianco, Gyula Kotek, Péter Zádori, Károly Zieber, Miklós S. Z. Kellermayer*
University of Pécs, Pécs, Hungary
Nanomanipulation of biomolecular systems with optical tweezers

10:40 Coffee break

Chairmen: László Kollár, Bernd Trathnigg

Lectures

11:00 L-18 *Xinghua Guo, Martin Trötzmüller, Ernst Lankmayr*
Technical University of Graz, Graz, Austria
Liquid chromatography-mass-spectrometry (LC-MS) for trace analysis: the detection specificity and its improvements

11:20 L-19 *Barna Kovács, László Kiss, Damir Tesanovic, Géza Nagy*
University of Pécs, Pécs, Hungary
Determination of diffusion coefficients for biosensing applications

11:40 L-20 *Michael Murkovic*
Technical University of Graz, Graz, Austria
Analysis of heat generated toxic substances in foods

12:00 L-21 *András Fittler, Zoltán Matus, Béla Kocsis, Lajos Botz*
University of Pécs, Pécs, Hungary
Chemical and microbiological aspects of the quantitative analysis of Amphotericin B

12:20 L-22 *Pál Perjési, Mónika Kuzma, Krisztina Fodor, Zsuzsanna Rozmer*
University of Pécs, Pécs, Hungary
Studies on in vitro antioxidant effect of salicylic acid and its hydroxylated metabolites

12:40 Lunch

14:00 Excursion to Mohács (historical places) and Villánykövesd (wine-tasting with dinner)

July2, 2008

9:30 Guided tour in Pécs starting from the Séta tér

12:00 Lunch

LIST OF POSTERS

P-01 *Hasnat Ahmed, Bernd Trathnigg*
Karl-Franzens University of Graz, Graz, Austria
Microwave assisted synthesis and characterization of poly (ethylene glycol)-b-poly(epszilon-caprolactone) by liquid chromatography under critical conditions

P-02 *Attila Almási, Emil Fischer, Pál Perjési*
University of Pécs, Pécs, Hungary
HPLC method for experimental quantitation of 4-nitrophenol and its metabolites from bile

P-03 *Fouzia Altaf, Qamar Abbas, Leo Binder*
Technical University of Graz, Graz, Austria
Investigation of copper corrosion by cyclic voltammetry and impedance spectroscopy

P-04 *Krisztina Babák*
University of Pécs, Pécs, Hungary
The alluvial deposits of flood-plains of Körös river during the river control

P-05 *Donata Bandoniene, Daniela Jöbstl, S. Obersriebnis, Thomas Meisel*
Montanuniversity Leoben, Leoben, Austria
ICP-MS determination of ree in different parts of pumpkin (Cucurbita pepo var. Styriaca) in relation to geographic origin

P-06 *Martina Blunder, Olaf Kunert, Antje Hüfner, Walter Fabian, Wolfgang Schühly, Rudolf Bauer*
Karl-Franzens University of Graz, Graz, Austria
Isolation and structure elucidation of Schisandra chinensis lignans and derivatives

P-07 *Orsolya Bouquet, Ildikó Kustos, Tamás Loránd, Ferenc Kilár, Béla Kocsis*
University of Pécs, Pécs, Hungary
Application of cell-chip method to rapid susceptibility testing

P-08 *Anita Bufa, Viktória Poór, Péter Gőcze, Ferenc Kilár*
University of Pécs, Pécs, Hungary
Endogenous urinary steroids in postmenopausal women with cervical cancer

P-09 *Efstathios Chatzistathis, Donata Bandoniene, Daniela Jöbstl, Thomas Meisel*
Montanuniversity Leoben, Leoben, Austria
Distribution of trace elements, especially rare erth elements, in different edible plant oils a mean for authentication

P-10 *Markus Damm, Gerald Rechberger, Manfred Kollroser, C. Oliver Kappe*
Karl-Franzens University of Graz, Graz, Austria
A kinetic evaluation of microwave-assisted derivatization procedures using hyphenated mass spectrometric techniques

P-11 *Fabrizio Donnarumma, Veronika Matzi, Alfred Maier, Ralph Herwig, Joachim Greilberger, Heinz Juan, Reinhold Wintersteiger*
Karl-Franzens University of Graz, Graz, Austria
Developement of a HPLC method for new antitumoral drugs using UV and fluorescence detection

P-12 *Anita Eberl, Tina Brückner, Sonja Heumann, Justyna Korpecka, Georg M. Gübitz*
Technical University of Graz, Graz, Austria
Mechanistic study of enzymatic and chemical hydrolysis of poly(ethylene-terephthalate) fabrics

P-13 *Alexander Fauland, Martin Trötzmüller, G. Fauler, Xinghua Guo, Ernst Lankmayr*
Technical University of Graz, Graz, Austria
SPE fractionation of phospholipids in bacterial cell-membrane and determination by HPLC-MS/MS

P-14 *Elisabeth Feizlmayr, Karin Wölkart, Peter Dittrich, Eckhard Böbler, Fritz Pinl, Andy Suter, Rudolf Bauer*
Karl-Franzens University of Graz, Graz, Austria
A pharmacokinetic study of Ginkgo preparations

P-15 *K. Fesko, C.Reisinger, J. Steinreiber, M.Schürmann, H.Griengl, W. Schnitzhofer, M. Vrsanska, P. Biely, A. Cavaco-Paulo, G. M. Gübitz, H. Weber*
Technical University of Graz, Graz, Austria
NMR spectroscopic analyses of enzyme catalyzed reactions

P-16 *Christina Gatschelhofer, Agnes Mautner, Franz Reiter, Michael R. Buchmeiser, Andreas Zimmer, Karin Wernig, Thomas R. Pieber, Frank M. Sinner*
Joanneum Research Forschungsgesellschaft mbH, Graz, Austria
Development of capillary-scale monolithic supports for on-line sample pre-treatment in nanomedical research

P-17 *Róbert Góra, Havlikova Danka, Milan Hutta*
Comenius University, Bratislava, Slovakia
Analysis and characterization of natural enviropolymers - humic substances and lignins - using off-line combination of liquid chromatography methods HPLC-SEC

P-18 *Barbara Gröblacher, Olaf Kunert, Wolfgang Schühly*
Karl-Franzens University of Graz, Graz, Austria
Isolation of dibenzocyclooctadiene-type lignans from Talauma gloriensis

P-19 *Juray Guzy, Janka Kubálková, Zenóbia Chavková, Mária Mareková, Vladimíra Tomečková, Pál Perjési*
PJ Safarik University, Kosice, Slovakia
Synthetic hydroxychalcones act as modulators of mitochondrial functions

P-20 *Györgyi Horváth, Péter Molnár, László Gy. Szabó, Erika Turcsi, József Deli*
University of Pécs, Pécs, Hungary
Separation and identification of carotenoids in some medicinal plants' flowers and inflorescence

P-21 *Heike Hödl, Kurt Plöschberger, Martin G. Schmid, Gerald Gübitz*
Karl-Franzens University of Graz, Graz, Austria
Enantioresolution of tryptophan derivatives on canine serum albumin stationary phases

P-22 *Tamás Huber, László Grama, Szabolcs Osváth, Judit Fidy, Miklós S. Z. Kellermayer*
University of Pécs, Pécs, Hungary
Topography and surface nanomechanics of living cells studied with AFM

P-23 *Violeta Ivanova, Ágnes Dörnyei, László Márk, Ferenc Kilár, Marina Stefova, Trajće Stafilov, Borimir Vojnoski*
University of Pécs, Pécs, Hungary
Analysis of wine and grape samples with MALDI-TOF-MS

P-24 *Éva Jámbor, Zoltán Patonai, Alexandra Váczy, László Márk*
University of Pécs, Pécs, Hungary
High-throughput mass spectrometric analysis of natural steroids

P-25 *Éva Jámbor, Réka Tihanyi, Tibor Gyula Szabó, Gyula Szabó, László Márk*
University of Pécs, Pécs, Hungary
Salivary proteomics of cleft palate patients by using MALDI-TOF-MS

P-26 *Éva Jámbor, Alexandra Váczy, Viktória Németh, László Márk*
University of Pécs, Pécs, Hungary
Analysis of oral tumor regulated salivary proteins

P-27 *Daniela Jöbstl, Donata Bandoniene, Thomas Meisel, Efstatios Chatzistathis*
Montanuniversity Leoben, Leoben, Austria
Identification of the geographic origin of pumpkin seed oil

P-28 *Árpád Karsai, Mátyás Kolsofszki, Ünige Murvai, Katalin Soós, Botond Penke, Miklós S.Z. Kellermayer*
University of Pécs, Pécs, Hungary
Beta-amyloid-based nano-networks explored with atomic force microscopy

P-29 *Roman Keimel, Anton Sadjak, R. Likar, Nina Gruber, Astrid Sottler, Reinhold Wintersteiger*
Karl-Franzens University of Graz, Graz, Austria
Compatibility of drugs in an implantable infusion system

P-30 *Gábor Keresztúry, Tom Sundius, Tamás Lóránd*
University of Pécs, Pécs, Hungary
Analysis of the vibrational spectra of some antibacterial aminoalcohols

P-31 *Ibolya Kiss, Ivett Bacskay and Attila Felinger*
University of Pécs, Pécs, Hungary
Comparison of the mass transfer kinetics in totally porous, superficially porous and nonporous reversed phase in liquid chromatography

P-32 *László Kiss, Damir Tesanovic, Géza Nagy, Barna Kovács*
University of Pécs, Pécs, Hungary
Determination of diffusion coefficients in gels with planar electrode arrangement

P-33 *Justyna Korpecka, Sonja Heumann, Anita Eberl, Georg M. Gübitz*
Technical University of Graz, Graz, Austria
Chemoenzymatic modifications of polymers in organic environment

P-34 *Anikó Kőnigné Péter, Tímea Dergez, Ferenc Kilár*
University of Pécs, Pécs, Hungary
Rapid and simple method for extraction and detection of gamma-butyrolactone

P-35 *Ivan Kron, Pál Perjési, Juray Guzy*
PJ Safarik University, Kosice, Slovakia
Acidity of some natural and synthetic chalcones

P-36 *Janka Kubálková, Juray Guzy, Zenóbia Chavková, Mária Mareková, Vladimíra Tomečková, Pál Perjési*
PJ Safarik University, Kosice, Slovakia
Activation of oxidative stress response by hydroxychalcones in mitochondria

P-37 *Sándor Kunsági-Máté, Koichi Iwata*
University of Pécs, Pécs, Hungary
Composition and stability of solvation shell of phenol derivatives in binary mixture of water and ethanol. A theoretical study

P-38 *Sándor Kunsági-Máté, Zsolt Csók, László Kollár*
University of Pécs, Pécs, Hungary
Permittivity-dependent complexation ability of tetranitro-calix[4]arenes towards para-substituted phenols

P-39 *Sándor Kunsági-Máté, Erika Ortmann, László Kollár, Martin Pour Nikfardjam*
University of Pécs, Pécs, Hungary
Entropy-driven complex formation of malvidin-3-O-glucoside with common polyphenols in ethanol-water binary solutions

P-40 *Sándor Kunsági-Máté, Sándor Bakonyi, László Kollár, Bernard Desbat*
University of Pécs, Pécs, Hungary
Effect of molecular environment on the coupling of molecular vibrations of p-cresol

P-41 *Géza Makkai, Andrea Buzády, János Erostyák*
University of Pécs, Pécs, Hungary
Sensitivity limits of Matrix Isopotential Synchronous Fluorimetry

P-42 *Muhammad Imran Malik, Nguyen Viet Cuong, Bernd Trathnigg*
Karl-Franzens University of Graz, Graz, Austria
Liquid chromatography at critical conditions (LCCC): practical applications

P-43 *Erika Marek, Piroska Niesz, Anita Bufa, Viktoria Poór, Francoise Raffalli-Matthieu, Ferenc Kilár*
University of Pécs, Pécs, Hungary
Detection of progesterone form culture media of Choriocarcinoma cells using gas chromatography-mass spectrometry

P-44 *Ákos Markovics, Géza Nagy, Barna Kovács*
University of Pécs, Pécs, Hungary
Sol-gel coated anodized alumina for sensor applications

P-45 *Agnes Mautner, Christina Gatschelhofer, Franz Reiter, Christoph Magnes, Michael R. Buchmeiser, Thomas R. Pieber, Frank M. Sinner*
Joanneum Research Forschungsgesellschaft mbH, Graz, Austria
Ion-exchange monolithic stationary phases prepared by ring-opening metathesis polymerization

P-46 *Marlene Monschein, Jacobo Neira Inglesias, Olaf Kunert, Franz Bucar*
Karl-Franzens University of Graz, Graz, Austria
Phytochemical investigation of Calluna vulgaris (l.) Hull using LC/PDA/ESI-MS analysis

P-47 *Susanne Hauser, Sonja Mödritscher, Chhanda Debnath, Astrid Ortner*
Karl-Franzens University of Graz, Graz, Austria
Determination of the antimalarial drug artemisinin by using modified carbon electrodes

P-48 *Lívia Nagy, Géza Nagy*
University of Pécs, Pécs, Hungary
Microsized amperometric biosensors for clinical diagnosis

P-49 *Lívia Nagy, Gergely Gyetvai, Géza Nagy*
University of Pécs, Pécs, Hungary
Recent results with SECM diffusion coefficient measuring

P-50 *Csilla Páger, Ferenc Kilár*
University of Pécs, Pécs, Hungary
Possibilities of determination of different dye substances using CIEF-MS analysis

P-51 *Beáta Peles-Lemli, László Kollár, Géza Nagy, Sándor Kunsági-Máté*
University of Pécs, Pécs, Hungary
The effect of vibration dynamics of SWCNTs on their ion and molecule transport

P-52 *Beáta Peles-Lemli, Péter Ács, László Kollár, Sándor Kunsági-Máté*
University of Pécs, Pécs, Hungary
Solubilization of SWCNTs: permittivity-dependent carrier property of aniline derivatives

P-53 *Tímea Pernyeszi, Imre Dékány*
University of Pécs, Pécs, Hungary
Surface fractal and structural properties of layered clay minerals monitored by small angle X-ray scattering and low temperature nitrogen adsorption experiments

P-54 *Friedrich W. Pichler, A. Ranz, Ernst P. Lankmayr*
Technical University of Graz, Graz, Austria
Analysis of volatile fatty acids to optimize the process of fermentative biogas production

P-55 *Karin Pickl, Olivia Schnitzer, Christoph Magnes, Thomas R. Pieber, Frank M. Sinner*
Joanneum Research Forschungsgesellschaft mbH, Graz, Austria
Sensitive insulin analysis in interstitial fluid: Influence of mobile phase additives on insulin b chain loading efficiency in cap-HPLC/MS2

P-56 *Elfriede Pittler, Martin G. Schmid, Gerald Gübitz*
Karl-Franzens University of Graz, Graz, Austria
Enantioseparation using dynamically coated reversed phase columns

P-57 *Andreas Ranz, Eveline Maier, Christian Trampitsch, Ernst P. Lankmayr*
Technical University of Graz, Graz, Austria
Brominated flame retardants - an extraction from polymers

P-58 *Andreas Ranz, Eveline Maier, Ernst P. Lankmayr*
Technical University of Graz, Graz, Austria
Determination of fatty acid derivatives in fuel oil

P-59 *Marzena Kaniewska, Stephanie Schweiger[1], Marianne Huber[1], Martin Pichler[2], Peter M. Abuja[2], and Martin Schmid*
Karl-Franzens University of Graz, Graz, Austria
Simultaneous determination of S-adenosyl-methionine and S-adenosyl-homocysteine in tissue by capillary electrophoresis

P-60 *Martin G. Schmid, Julia Koidl, Iris Mühlhauser, Gerald Gübitz*
Karl-Franzens University of Graz, Graz, Austria
Chiral separation of alpha-hydroxy acids by capillary electrochromatography using Ristocetin A as chiral selector

P-61 *Margot Schober, Veronika Matzi, Alfred Maier, Ralph Herwig, Joachim Greilberger, Giuseppe Martano, Heinz Juan, Reinhold Wintersteiger*
Karl-Franzens University of Graz, Graz, Austria
Determination of two new anti-cancer agents in plasma via a double-cartridge-SPE-HPLC-method

P-62 *Maria Suppan, Christoph Magnes, Barbara Daum, Peter Stadler, Thomas R. Pieber, Frank M. Sinner*
Joanneum Research Forschungsgesellschaft mbH, Graz, Austria
Validated analytical method for determination of L-cystine, N-acetyl-L-cysteine, L-cysteine and rearrangement products in amino acid solutions by LC/MS/MS

P-63 *Kornélia Szabó, Géza Nagy, László Kollár, Sándor Kunsági-Máté*
University of Pécs, Pécs, Hungary
Increasing the water solubility of o-hydroxy-acetophenone by hydrotropic p-toluene sulfonate

P-64 *Dávid Szatmári, Tamás Huber, Viktória Németh, Veronika Kollár, László Grama, Miklós Kellermayer*
University of Pécs, Pécs, Hungary
Dissecting the mechanosensor functions of titin

P-65 *Mónika Szili, Géza Nagy, Barna Kovács*
University of Pécs, Pécs, Hungary
Polyluminol based biosensor for H2O2

P-66 *Martin Trötzmüller, Ernst P. Lankmayr*
Technical University of Graz, Graz, Austria
Optimization of extraction methods for antioxidants from polyolefins

P-67 *Martin Trötzmüller, Xinghua Guo and Ernst P. Lankmayr*
Technical University of Graz, Graz, Austria
Features and origins of common chemical background ions in ESI-HPLC-MS/MS

P-68 *Thomas Ulrich, Gerd-Joachim Krauss, Dirk Wesenberg, Martin G.Schmid, Gerald Gübitz*
Karl-Franzens University of Graz, Graz, Austria
Enantioselective chromatographic and capillary electrophoretic determination of the ß-2-sympathomimetic fenoterol for pharmakokinetic studies

P-69 *Péter Vajda, Szymon Bocian, Bogusław Buszewski, Attila Felinger*
University of Pécs, Pécs, Hungary
Modelling of overloaded band profiles of organic modifiers on C-18 RPLC column

P-70 *Beata Veliká, Ivan Kron*
PJ Safarik University, Kosice, Slovakia
Antioxidant properties of hydroxbenzoic acid derivatives

P-71 *Hedda K. Weber, Moritz Leschinsky, Petra Wollboldt, Gerhard Zuckerstätter, H.Sixta*
Kompetenzzentrum Holz GmbH, Lenzing, Austria
Contemporary lignin analytics by NMR-spectroscopy

P-72 *Ute Widowitz, E.M. Wenzig, S. Chrubasik, E. Knauder, F. Bucar, R. Bauer*
Karl-Franzens University of Graz, Graz, Austria
Phytochemical and pharmacological investigations of rose hip powders

P-73 *Alam Zeb, Michael Murkovic*
Technical University of Graz, Graz, Austria
Thin layer chromatographic analysis of carotenoids

P-74 *Lyubomir Georgiev, Tamara Pajpanova, Ferenc Kilár*
"Neofit Rilski" University, Blagoevgrad, Bulgaria
Optimization of capillary isoelectric focusing for acidic and highly basic compounds

P-75 *Balázs Kiss, Árpád Karsai, Miklós S.Z. Kellermayer*
University of Pécs, Pécs, Hungary
Elasticity and force-induced structural transitions of individual desmin intermediate filaments

P-76 *Nasim Ghasemzadeh, Fred Nyberg, Stellan Hjertén*
Uppsala University, Uppsala, Sweden
Simple test for the selectivity of the Artificial gel antibodies by fluorometry

ABSTRACTS

LECTURES

L-1 - L-22

L-01

MOLECULAR BACKGROUND OF PSYCHIATRIC DISEASES: PROTEOMIC STUDIES

Tamás Janáky[1], Zoltán Szabó[1], Szeliné Judit Szomor[2,3], Attila Csorba[2,3], Éva Szegő[4], Katalin Kékesi[4], Gábor Juhász[4], György Lévay[5]

(1) University of Szeged, Department of Medical Chemistry, 6720 Szeged, Dóm tér 8, Hungary, janaky@mdche.szote.u-szeged.hu
(2) Kromat Ltd., 1124 Budapest, Sirály utca 3., Hungary
(3) South-Plain Neurobiologic Research Centre, 6725 Szeged, Szikra u. 2., Hungary
(4) Eötvös Lóránd University, Institute of Biology, 1117 Budapest, Pázmány Péter sétány 1/c, Hungary
(5) EGIS Pharmaceuticals PLC, 1106 Budapest, Keresztúri út 30-38., Hungary

Psychiatric diseases (anxiety and depression) represent a significant portion of neurological disorders. The altered "tuning" of the cells could be one of the reasons of these diseases. The main goal of our study is to understand the mechanisms of these diseases at molecular level, involving genomic and proteomic changes in brain. These changes may occur partly triggered by the signal systems, partly due to direct changes in gene expression. The newest developments of functional genomics and proteomics may permit following these changes.

As the animal model of the anxiety, mice bred selectively for high-anxiety-related behavior for more than 30 generation have been selected. Qualitative and quantitative differences in the protein composition in the brain of these anxious and control animals were revealed by 2D differential gel electrophoresis. 93 proteins having different expressions in two groups were identified by LC/MS/MS and bioinformatics.

The „Protein Expression Systems" from Waters Inc. was developed to determine different expression of proteins in fairly complex mixtures. We have used this system for qualitative and quantitative determination of proteins in mouse's brain: the process involved separation of peptides from digested brain tissue by 1D nanoUPLC followed by on-line QTOF-MS/MS analysis. Among the identified 350 proteins about 1/3 of those proteins were detected which were found differentially expressed in the above mentioned experiment.

Protein expression in mouse brain (anxious, „brave" and control) –followed by administration of an anxiolitic– was evaluated using this 1D-LC-MS method.

In recent talk we present those separation, mass spectrometric and bioinformatic methods which lead to proteomic characterization of proteins may play a role in the development of psychiatric diseases.

L-02

VARIATION OF FLAVONOID PROFILES IN MEDICINAL PLANTS COLLECTED AT DIFFERENT ALTITUDES

Gudrun Rieger, Franz Bucar

Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University of Graz, Universitätsplatz 4/1, A-8010 Graz, Austria
franz.bucar@uni-graz.at

Flavonoids are regarded as important secondary metabolites in food and medicinal plants due to their health promoting and therapeutic activities [1,2].
The concentrations of polyphenol compounds in the plant tissues vary apart from genetic factors or age of the plant according to numerous exogenous factors like environmental parameters, time of harvest, infestation with microorganisms and damage caused by different pests including competition with other individuals or species, some of these environmental factors depend on sea level [3,4].
In this study the altitudinal variation and its effect on the content of flavonoids in three traditional herbal medicinal plants was investigated for the first time. Herbs of *Calluna vulgaris* (L.) HULL, flowers and fruits of *Sambucus nigra* L. and berries of *Vaccinium myrtillus* L. collected in the Naturpark Sölktäler (Austria) were extraced using Accelerated Solvent Extraction (ASE). Identification and quantification of flavonoids in the polar extracts (methanol 80%; v/v) was achieved by means of RP-HPLC/PDA and/or LC/PDA/MS with external standards.
While the variation of the content of taxifolin-3-*O*-glucoside in *C. vulgaris* was too large to deduct an association with altitude, it seems that the concentration especially of flavonol-3-*O*-glycosides with adjacent hydroxyl groups in ring B in *C. vulgaris* and *S. nigra* rise with increasing altitude. One reason for that might be the influence of increasing UV-B-radiation with increasing sea level [4].
Anthocyanins from both the berries of *S. nigra* and *V. myrillus* ocurred in decreasing amounts with rising altitude.
The results of the investigations on the radical scavenging capacity of the extracts using the DPPH-assay reflected these findings with *C. vulgaris* showing the best activity in our study.

Acknowledgements
The authors are grateful to the Naturpark Sölktäler for providing plant material, to the Faculty of Natural Sciences/Karl-Franzens-University of Graz and also to the Gandolph-Doelter and Dr. Heinrich-Jörg foundations for financial support.

References
[1] Boehm, H., Boeing, H., Hempel, J., Raab, B., Kroke, A. *Z. Ernaehrungswiss.* **37** (1998) 147-163.
[2] Hooper, L., Cassidy, A. *J. Sci. Food Agric.* **86** (2006)1805-1813.
[3] Körner, C. *Alpine Plant Life*. Springer, Berlin, Heidelberg (2003).
[4] Blumthaler, M., Ambach, W., Ellinger, R. *J. Photochem. Photobiol. B* **39** (1997) 130-134.

L-03

SIMPLE FABRICATION OF FRITLESS CHROMATOGRAPHIC MICROCHIPS

Attila Gáspár[1,2], István Bácsi[2], Menake E. Piyasena[2], Frank A. Gomez[2]

(1) Department of Inorganic and Analytical Chemistry, University of Debrecen, 4032 Debrecen, Egyetem tér 1, Hungary, gaspara@tigris.unideb.hu
(2) Department of Chemistry and Biochemistry, California State University, Los Angeles, 5151 State University Drive, Los Angeles, CA 90032

Despite the high demand for miniaturized chromatographic techniques, few chip-based chromatographic systems compared to chip-based capillary electrophoretic (CE) devices are known, mainly due to technical problems inherent in the former. Although CE is a highly efficient analytical method, liquid chromatography (LC) is more frequently used in separation applications. Hence, the development of new miniaturized lab-on-a-chip devices integrating LC is warranted. Herein, we describe the design of a disposable and inexpensive microfluidic chip, fabricated from poly(dimethylsiloxane) (PDMS), incorporating conventional chromatographic reversed-phase silica beads (C18) without the use of frits or permanent physical barriers, tapers or restrictors. The packing of C18 modified silica beads into the microfluidic channels is made possible by the hydrophobic nature and excellent elasticity of PDMS. Reasons for the unexpectedly easy stabilization of the chromatographic packing within the channels are detailed. The possible analytical utilization of a recently studied paper chromatographic procedure in PDMS chip is also demonstrated.

L-04

NUMERICAL CALCULATIONS OF THE REVERSIBLE AND IRREVERSIBLE INTERACTION OF AN ANALYTE TO THE WALL OF THE SEPARATION CHANNEL IN CE AND MICROCHIP ELECTROPHORESIS. ARE THESE INTERACTIONS THE CAUSE OF THE LOW REPRODUCIBILITY OF CE-MS EXPERIMENTS WITH PROTEINS?

Viktor Farkas[1,2], Melinda Rezeli[1,2], Ákos Végvári[4], Ferenc Kilár[2,3], Stellan Hjertén[1]

(1) Department of Biochemistry and Organic Chemistry, BMC, Uppsala University, SE-751 23 Uppsala, Sweden
(2) Department of Analytical and Environmental Chemistry, University of Pécs, H-7624 Pécs, Hungary
(3) Institute of Bioanalysis, University of Pécs, H-7624 Pécs, Hungary
(4) Division of Clinical Protein Science, BMC, Lund University, SE-221 00 Lund, Sweden

Miniaturization of analytical separation methods offers several advantages, including high resolution.
A prerequisite is that the analytes do not interact reversibly or irreversibly with the wall of the separation channel, for instance in capillary and microchip electrophoresis.
For a quantitative treatment of such interactions we have derived equations (1) to decide whether the analyte is *reversibly* or *irreversibly* adsorbed to the channel wall (2) to determine the *reversible* adsorption quantitatively in the form of the variance and (3) to determine the *irreversible* adsorption quantitatively in percent of the amount of the applied analyte. These equations cannot in a straightforward way be based on electropherograms recorded by the conventional, *stationary* detector, because these separation patterns are apparent, particularly in experiments where the analytes are transported not only by electrophoresis, but also by a fast electroosmotic flow (EOF).
However, under very special experimental conditions this type of detector gives true separation parameters and electropherograms which permit quantitative determinations of these interactions (these conditions are discussed).
A perfect electroosmotic flow in open channels has a plug-like profile, i.e. it cannot *per se* change the shape of the zone. Consequently, it can affect the resolution only by increasing or decreasing the migration velocity at a given separation distance, provided that the analyte does not adsorb onto the wall of the channel.
An example of the practical use of EOF is the transport of electrophoretically separated zones into a mass spectrometer. The power of this method is hampered by the wide variations often occurring between different laboratories in quantitative measurements of a standardized sample, maybe partially caused by disregarding the above adsorption onto the wall of the separation channel (the equations derived herein permit quantitative calculations of these losses) or/and by employing the conventional, erroneous formula for the calculation of the amount of the analyte in a zone (the correct equation is presented).

L-05

ENANTIOSEPARATION OF N-PROTECTED β-AND γ-AMINO ACIDS FOR BIOCATALYTIC NITRILE HYDROLYSIS

Margit Winkler, Norbert Klempier

Institute of Organic Chemistry, Graz University of Technology, A-8010 Graz, Stremayrgasse 16, Austria
klempier@tugraz.at

The enantioseparation of a series of structurally related carbo- and heterocyclic *N*-protected β-/γ-amino acids and β-/γ-amino amides - products from enzymatic nitrile hydrolysis - as well as and β-/γ-amino nitriles - starting materials for the biohydrolysis - by using six different commercially available chiral stationary phases (CSPs) is accomplished. The separation characteristics of all CSPs and their abilities for monitoring the biotransformation of *N*-protected β-/γ-amino acid derivatives are discussed based on the chromatographic data of all separated compounds.

Keywords: enantioseparation, biotransformation, β-/γ-amino acids, Chirobiotic R, Crownpak CR(+), Chiralcel OD-H, Chiralcel OJ, Chiralpak AD-H, Chiral AGP

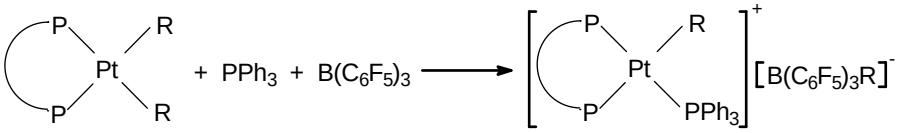
L-06

NMR INVESTIGATIONS OF THE PLATINUM-ALKYL/ARYL - $B(C_6F_5)_3$ 'IN SITU' SYSTEMS AS CATALYSTS

László Kollár[1], László Jánosi[1], Tamás Kégl[2]

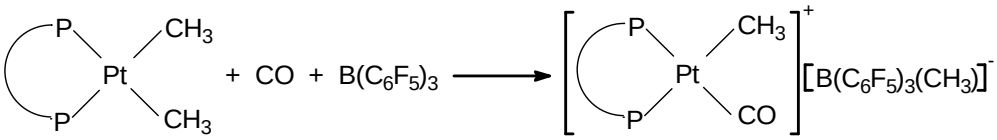
(1) Institute of Chemistry, Department of Inorganic Chemistry, University of Pécs, Ifjúság u. 6., 7624 Pécs, Hungary, kollar@ttk.pte.hu
(2) Institute of Chemistry, Department of Organic Chemistry, University of Pannonia, Egyetem u. 8., 8200 Veszprém

The dialkyl/diaryl-platinum complexes (PtR_2(*bdpp*), where *bdpp* stands for (*2S,4S*)-2,4-bis(diphenylphosphino)pentane) were reacted either with $B(C_6F_5)_3$, BPh_3 or BF_3. In the presence of PPh_3 *(eq. 1)* or carbon monoxide *(eq. 2)* cationic species [1] with a general formulae $[PtR(L)(bdpp)]^+$ (L=PPh_3, CO) were formed exclusively. The catalytic precursors and potential intermediates were characterised by ^{31}P NMR spectroscopy. Couplings constants ($^1J(^{31}P,^{195}Pt)$) of diagnostic value were used as most important features for identification.



R = Me, Ph, 2-Thioph

(eq. 1)



(eq. 2)

The ability of boron additives to provide vacant coordination site at the platinum [2] made these systems suitable as *hydroformylation catalysts*. Enantioselective hydroformylation was carried out in the presence of *in situ* catalysts formed from Pt(alkyl/aryl)$_2$(bdpp) and $B(C_6F_5)_3$ or BF_3. DFT/PCM calculations reveal an S_N2-type reaction mechanism for the alkyl/aryl ligand abstraction.

References

[1] Tóth, I.; Elsevier, C.J.; Kégl, T.; Kollár, L. *Inorg. Chem.* **33** (1994) 5708-5712.
[2] Jánosi, L.; Kégl, T.; Kollár, L. *J. Organomet. Chem.* **693** (2008) 1127-1135.

L-07

THE APPLICATION OF HPLC-MASS SPECTROMETRY FOR THE ELUCIDATION OF SELENIUM METABOLISM IN MAMMALS

Doris Kühnelt, Dijana Juresa, Norbert Kienzl, Kevin A. Francesconi

Institute of Chemistry, Analytical Chemistry, Karl-Franzens-University, Universitaetsplatz 1, 8010 Graz, Austria, doris.kuehnelt@uni-graz.at

Selenium is of particular scientific interest because of its essentiality, claimed health benefits, and – on the other hand - toxicity at quite modest intakes. Investigations into selenium urinary metabolites, particularly after controlled intake of defined selenium compounds, can provide insight into selenium metabolism in the body. Analytical techniques employing coupling of high performance liquid chromatography (HPLC) to elemental and molecular mass spectrometry have significantly advanced this field during the past decade.

Especially speciation of selenium compounds in urine of unsupplemented subjects – usually containing less than 30 µg Se/L distributed among several selenium species - requires the use of detectors with low detection limits. Recent developments and results in the field of speciation analysis of selenium urinary metabolites achieved with HPLC coupled to mass spectrometry will be presented. These include the application of HPLC coupled to inductively coupled plasma mass spectrometry (ICPMS), vapor generation ICPMS, and electrospray mass spectrometry. Results obtained for urinary selenium metabolites with and without selenium supplementation will be discussed with respect to their significance in adding to our knowledge about selenium metabolism.

Finally, remaining analytical challenges associated with the determination of selenium species in urine and other body liquids will be addressed.

L-08

CAPILLARY ELECTROPHORESIS CHIPS FOR SCREENING OF ENDOTOXIN CHEMOTYPES FROM WHOLE-CELL LYSATES

Anikó Kilár[1], Zoltán Péterfi[1], Eszter Csorba[2], Zoltán Szabó[3], Ferenc Kilár[2], Béla Kocsis[1]

(1) Department of Medical Microbiology and Immunology
(2) Institute of Bioanalysis
(3) Institute of Biochemistry and Medical Chemistry
Faculty of Medicine, University of Pécs, Szigeti út 12, 7624 Pécs, Hungary

A fast microchip electrophoresis method was developed to analyse and differentiate bacterial endotoxins directly from whole-cell lysates after removal of the proteinaceous components with proteinase K digestion. The method is based on the visualization of endotoxins by the interaction with sodium dodecyl sulphate and then a fluorescent dye. The lipopolysaccharide (LPS) profiles can be directly evaluated from digested bacterial cells, and the electrophoresis patterns are very closely resembled to those of the purified LPSs, and the *R* and *S* chemotypes can be used to assign the strains. The method has been found to be useful in preliminarily structural characterization of endotoxins extracted from as small volume as one milliliter cultures of a large number of mutants.

The work was supported by the grants GVOP-3.2.1-0168, RET 008/2005 and OTKA-NKTH-NI- 68863.

L-09

PROTEOGLYCANOMICS: THE SUGAR SIDE OF DRUG DEVELOPMENT

Andreas Kungl

Institute of Pharmaceutical Sciences, University of Graz, Universitaetsplatz 1, A-8010 Graz, Austria; and ProtAffin Biotechnologie AG, Reininghausstr. 13a, A-8020 Graz, Austria

Glycosylation is the most frequent post-translational protein modification and contributes significantly to the function of proteins depending on the type of glycosylation. Especially O-linked glycan structures like the glycosaminoglycans (GAGs) themselves are considered to constitute the major functional part of the glycoconjugate which is therefore termed proteoglycan in order to differentiate them from convetional glycoproteins. The term proteoglycanome relates to the interaction network of GAGs with a multitude of proteins, a functional network which forms the base of many different (patho-)physiological conditions. We will present data on how the proteoglycanome can be experimentally addressed and how this knowledge can be translated into protein-based therapeutic approaches. Since glycans are generally not amenable for synthetic chemistry, we have developed protein-based glycan antagonists. We have sucessfully tested this new class of biologics in animal models of inflammatory and oncological disorders.

L-10

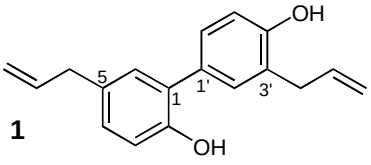
SEPARATION OF BIPHENYL-TYPE LIGNANS FROM *MAGNOLIA GRANDIFLORA* BY USING FAST CENTRIFUGAL PARTITIONING CHROMATOGRAPHY (FCPC)

Christine Grabner, Rudolf Bauer, Wolfgang Schühly

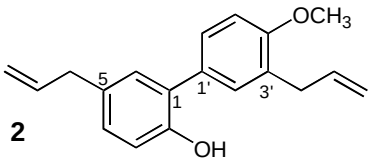
Institute of Pharmaceutical Sciences, Pharmacognosy,
Universitätsplatz 4, 8010 Graz, Austria
wolfgang.schuehly@uni-graz.at

Honokiol (**1**) and magnolol (**3**) are biphenyl-type lignans and are considered to carry the pharmacological activity of *Magnolia* bark (*houpo*) used in Traditional Chinese Medicine [1]. The seeds of *Magnolia grandiflora* L. (Magnoliaceae), a tree native to the Southeastern United States and Mexico, are a good source of methylhonokiol (**2**), together with honokiol and magnolol [2]. In order to isolate compounds **1** to **3** by liquid-liquid partitioning from the oily seed extract, fast centrifugal partitioning chromatography (FCPC) was applied using solvent mixtures containing water, ethanol, hexane and ethyl ether.

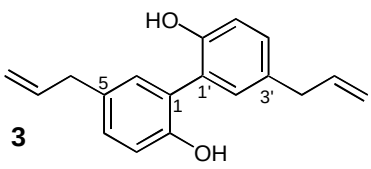
We herein present a fast and reproducible method for the separation of the three major biphenyl-type phenyl propanoids from *Magnolia grandiflora* L., which allows to separate the three compounds in a single run of about 60 min.



1 Honokiol



2 Methylhonokiol



3 Magnolol

References

[1] Maruyama Y, Ikarashi Y, Kurihara H. Bioactivity and pharmacological aspects of Magnolia. In: Sarker SD, Maruyama Y, editors. *Magnolia. Medicinal and aromatic plants – industrial profiles*. London, New York: Taylor & Francis. 2002. P. 75-127.

[2] Schühly W, Khan I, Fischer NH. The ethnomedicinal uses of Magnoliaceae from the southeastern United States as leads in drug discovery. *Pharm Biol* **39** (2001) 63-69.

L-11

STUDY OF THE MOLECULAR ORGANIZATION AT THE AIR/WATER INTERFACE USING PMIRRAS, SURFACE PRESSURE AND BAM METHODS

Dominique Cavagnat[1], Joelle Mascetti[1], Daniel Blaudez[2], Sabine Castano[3], Bernard Desbat[3]

(1) Institut des Sciences Moléculaires, University of Bordeaux1, CNRS 351, cours de la Libération, F-33405, Talence, France
(2) Centre de Physique Moléculaire et Hertzienne, University of Bordeaux1, CNRS 351, cours de la Libération, F-33405, Talence, France
(3) Chime et Biochimie des Molécules et Nanosystèmes, University of Bordeaux1, CNRS, ENITAB, 2 rue Robert Escarpit, F-33607, Pessac, France

In situ characterization of monomolecular films spread at the air/water interface is a fundamental issue of particular significance to the study of model biological membranes. The interest of studying monomolecular films at the air/ water interface stems from the wide latitude in choosing the composition and physical state of the molecules in the monolayer. Processes such as insertion of proteins and peptides into phospholipid monolayers can be followed.
In this talk we want to show the interest to coupled PMIRRAS (Polarization Modulation Infrared Reflection Spectroscopy), [1,2] which is a molecular spectroscopy, with the BAM (Brewster Angle Microscopy) and Surface pressure methods to obtain informations on the structure and organization of the monolayers at the air/water interface. Many exemples on fatty acids, phospholipids, peptides, protein and cyclodextrin will be presented showing the possibility to determine the orientation of the molecules and their mutual interaction.

References

[1] Blaudez, D.; Turlet, J.M.; Dufourcq, J.; Bard, D.; Buffeteau, T.; Desbat B. J. Chem. Soc.Faraday Trans., **1996**, 92, 525
[2] Gallant, J.; Desbat, B.; Vaknin, D.; Salesse, C. Biophys. J., **1998**, 75, 2888-2899

L-12

MULTIRESIDUE DETERMINATION OF SEVEN QUINOLONES ANTIBIOTICS IN GILTHEAD SEABREAM USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

Victoria Samanidou[1], E. Evaggelopoulou[1], Martin Trötzmüller[2], Xinghua Guo[2], Ernst P. Lankmayr[2]

(1) Aristotle University of Thessaloniki, Laboratory of Analytical Chemistry, GR-54124 Thessaloniki, Greece,samanidu@chem.auth.gr
(2) Graz University of Technology, Institute of Analytical Chemistry & Radiochemistry, Technikerstraße 4, 8010 Graz, Austria

The widespread and unrestricted use of antibiotics in aquaculture to prevent bacterial infections, leads to remaining amounts in the aquatic environment. Their extensive administration to fish, destined for human consumption, has become a serious problem because their residues in fish tissue and fish products may be directly toxic or be the source of resistant human pathogens representing a possible risk to human health. Quinolones constitute the main group of antibiotics used in veterinary medicine for therapeutic purposes for food-producing animals. The mechanism of action of quinolones is bactericidal. Quinolones are used in fish farm industries in cases of pulmonary, urinary and digestive infections as they act by inhibiting bacterial DNA-gyrase.

This paper presents an analytical method for the multiresidue determination of seven quinolones: ciprofloxacin, enrofloxacin, sarafloxacin, danofloxacin, oxolinic acid, nalidixic acid and flumequine, in gilthead seabream *(Sparus Aurata).* The procedure involves a rapid and efficient pre-treatment by solid-phase extraction, followed by the sensitive and selective determination of all compounds in a single run using LC–ESI-MS/MS.

The separation was achieved on a Perfectsil ODS-2, 5 μm, 250 x 4 mm, analytical column (MZ Analysentechnik). A gradient elution program was followed using a mixture of 0.2 % formic acid, methanol and acetonitrile within 30 min. The sample was edible muscle and skin from gilthead seabream. The tissue samples were minced and homogenized using a kitchen blender. Extraction of quinolones was achieved by 0.1 M NaOH. Extracts were purified by SPE using Oasis HLB cartridges preconditioned with 2 mL MeOH and 2 mL H_2O. The elution was performed with 1.5 mL 0.1 % TFA in ACN and 0.5 mL ACN and the eluent was evaporated under a gentle steam of N_2 at 45 ^oC. To the dry residue 200 μL of a mixture (50 % H_2O, 40 % ACN and 10 % MeOH, v/v) containing 0.2 % HCOOH in the final solution, were added. After vortex mixing for 5-10 seconds, the samples were sonicated for 20 min in the ultrasonic bath. This procedure was repeated twice to enhance solubility and recovery of the analytes. Subsequently the samples were filtered by PTFE syringe filter 0.22 μm in order to obtain a clear solution for LC-MS/MS analysis.

Multiple reaction monitoring (MRM) was used for selective detection of each quinolone. Accuracy was evaluated through recovery studies at three different fortification levels. Satisfactory results were found, with mean recoveries between 99.6-124.0 % for the selected levels. The repeatability had RSD values lower than 20 %. The method presents satisfactory results of linearity, precision and limits of quantification much lower than the MRLs established by the European Union for quinolones in fish tissues (6-8 μg/kg). Selectivity was evaluated extracting and analysing blank tissue samples. The absence of any signal at the same elution time as the selected antibiotics indicates that there were no matrix interferences.

L-13

INTERACTION OF FORMIN WITH SPIN-LABELED ACTIN

Tünde Kupi[1], Pál Gróf[2], Miklós Nyitrai[1], József Belágyi[1]

(1) Institute of Biophysics, Medical Faculty, University of Pecs, Pecs
(2) Institute of Biophysics, Semmelweis Medical University, Budapest

Actin, the main component of the thin filament of muscle and of the cytoskeletal system, was studied by EPR spectroscopy in interaction with formin. Actin was isolated from back and leg muscles of rabbit and was spin-labeled either with maleimide (MSL) or fluoro-dinitroanilino-proxyl nitroxide (FDNA-SL). The MSL probe molecules were attached to Cys 374 residue of subdomain I, the FDNA-SL to Lys 61 residue of subdomain II. The rotational correlation times in monomer form of actin were 18 ns and 8-9 ns, reporting that the FDNA-SL has some residual motion with respect to protein backbone. In F-form of FDNA-SL-actin the EPR spectrum was superposition of two spectra implying that either the label attached to different residues or the attached reporter molecules reflected two conformations.
In partially oriented macroscopic samples the EPR spectra showed orientation dependence with respect to the laboratory magnetic field. Spectrum analysis by computer manipulation suggests that in the case of MSL label the orientation could be modeled in terms of a Gaussian distribution with a center of 36° with respect to filament long axis. Using the FDNA-SL, the spectrum exhibited two components in both parallel and perpendicular orientation; their fractions depended on the orientation. The hyperfine splitting constants of the components were $2A'_{zz}$ = 5.37 mT and 6.53 mT, respectively.
Addition of formin to actin affected the motion of probe molecules both in conventional and saturation-transfer EPR time-domain, and the changes were function of the molar ratio of formin to actin.

MONOLITHIC CAPILLARY COLUMNS PREPARED BY RING-OPENING METATHESIS POLYMERIZATION AND THEIR APPLICATION IN MEDICAL RESEARCH

Frank M. Sinner[1], Christina Gatschelhofer[1], Agnes Mautner[1], Franz Reiter[1], Michael R. Buchmeiser[2], Andreas Zimmer[3], Karin Wernig[3], Thomas R. Pieber[1,4]

(1) Institute of Medical Technologies and Health Management, JOANNEUM RESEARCH, Auenbruggerplatz 20, A-8036 Graz, Austria
(2) Leibniz Institute for Surface Modification e. V., Permoserstr. 15, D-04318 Leipzig, Germany, and Institute of Technical Chemistry, University Leipzig, Linnéstr. 3, D-04103 Leipzig, Germany
(3) Department of Pharmaceutical Technology, Institute of Pharmaceutical Sciences, University of Graz, Schubertstr. 6, A-8010 Graz, Austria
(4) Department of Internal Medicine, Diabetes and Metabolism, Medical University Graz, Auenbruggerplatz 15, A-8036 Graz, Austria
frank.sinner@joanneum.at

The rapid pace of development in environmental science, the pharmaceutical industry and in the life sciences is placing great demands on the performance level of analytical techniques. Samples are becoming increasingly complex and there is still an expectation that the analysis should be as fast, as cheap and as sensitive as possible. In this regard, monoliths have already become powerful tools in separation science due to their unique structural properties combining high porosity and flow-rate independent efficiency [1].
To date, a variety of materials and synthetic routes have been used for monolith preparation. Sinner and Buchmeiser for instance introduced the use of ring-opening metathesis polymerization (ROMP) for the preparation of monolithic stationary phases [2]. This new class of monolithic separation media is based on norborn-2-ene (NBE) and 1,4,4a,5,8,8a-hexahydro-1,4,5,8-*exo*,*endo*-dimethanonaphthalene (DMN-H6) which are copolymerised with a Grubbs-type initiator in the presence of a mixture of toluene and 2-propanol. Analytical-scale ROMP-derived monolithic columns are already well characterized. The increasingly stringent requirements of the field of bioanalysis are, however, strongly driving a trend towards further downscaling of column inner diameter. We therefore extended the concept of ROMP to the preparation of monoliths in capillary format [3,4] for capillary liquid chromatography (CLC) and for capillary electro-chromatography (CEC).
This contribution will cover the synthesis of ROMP-derived monolithic capillary columns for reversed phase and ion-exchange chromatography in different column dimensions ranging from 530 to 50 µm inner diameter. For both column types the effect of the polymerization procedure on separation performance and resulting monolithic structures will be discussed. Furthermore, we will present the utility of capillary-scale ROMP monoliths as analytical and pre-concentration columns, demonstrating their potential in medical and nanomedical research.

References

[1] Tanaka, N.; Kobayashi, H.; Ishizuka, N.; Minakuchi, H.; Nakanishi, K.; Hosoya, K.; Ikegami, T. *J.Chromatogr.A* **2002,** *965*, 35-49
[2] Sinner, F. M.; Buchmeiser, M. R. *Angew.Chem.* **2000,** *112*, 1491-94.
[3] Gatschelhofer, C.; Magnes, C.; Pieber, T. R.; Buchmeiser, M. R.; Sinner, F. M. *J.Chromatogr.A* **2005,** *1090*, 81-89.
[4] Sinner, F. M.; Gatschelhofer, C.; Mautner, A.; Magnes, C.; Buchmeiser, M. R.; Pieber, T. R. *J.Chromatogr.A* **2008,** *in press.*]

L-15

CHARACTERIZATION OF STATIONARY PHASES FOR REVERSED-PHASE LIQUID CHROMATOGRAPHY BY SOLVENT AND SOLUTE ADSORPTION PHENOMENA

Attila Felinger[1], Péter Vajda[1], Szymon Bocian[2], Bogusław Buszewski[2]

(1) Department of Analytical and Environmental Chemistry, University of Pécs,,Ifjúság útja 6, H-7624 Pécs, Hungary
(2) Faculty of Chemistry, Department of Environmental Chemistry and Ecoanalytics, Nicolaus Copernicus University, 7 Gagarin Street, PL-87100 Torun, Poland

The understanding of the adsorption of solvents used as mobile phase components is essential for the explanation of solute retention mechanism on the stationary phases in reversed phase liquid chromatography. The commercial stationary phases used in reversed-phase high performance liquid chromatography (RP HPLC) have different chemical properties. In the structure of the bonded phase, three types of adsorption centers may be observed: hydrophobic organic bonded ligands, residual silanols and polar function groups. The properties of the stationary phase depend on parameters such as coverage density, coverage homogeneity, the length of organic ligands and the type of the functional groups in the ligand structure.

Our work is focused on the retention behavior of the commonly used solvents: methanol, acetonitrile and tetrahydrofuran on the stationary phases in RP HPLC. The adsorption isotherms of organic modifiers on chemically bonded stationary phases were studied, using packed columns containing organic ligands with different function groups: amino, amido, cholesteryl, phenyl, cyano and C4 and C18 alkyl. Another series of C18 stationary phases with different coverage density was also studied. The excess isotherms of the organic modifier from water were measured using the minor disturbance method. The solvent adsorption measurement from pure water as a mobile phase was also carried out by the inverse method.

The relative adsorption of the organic modifier informs about the heterogeneity of the adsorbent surface, the accessibility of the residual silanol groups to the mobile phase components and preferential solvatation which is essential for separation selectivity. The comparison of our results with the adsorption of solvents on the alkyl modified adsorbents let us to describe the effect of polar groups on the adsorption of the organic modifiers. Our results demonstrate how the function groups modified the properties of the homogeneous hydrophobic adsorbent and how the properties of alkyl phase changes with the coverage density of bonded ligands.

To further characterize the effect of surface coverage on retention properties, the adsorption isotherms of phenol and aniline were measured by frontal analysis and by inverse method on a series of C18 columns. The affinity energy distribution calculation may be useful for the characterization of surface heterogeneity.

L-16

PREDICTION OF RETENTION IN LIQUID CHROMATOGRAPHY OF POLYMERS

Bernd Trathnigg

Institute of Chemistry , Faculty of Natural Sciences, Karl-Franzens University Graz
Heinrichstrasse 28, A-8010, Graz, Austria

In chromatography of polymers, retention is determined by the characteristic volumes of the column (pore volume V_p and interstitial volume V_i), the pore diameter D, and the interaction parameter c. While the influence of the pore diameter is predominant in size exclusion chromatography (SEC), the key parameters describing retention in liquid adsorption chromatography (LAC) are the interaction parameter c and the pore surface S_p of the column, which is related to the pore volume V_p and pore diameter D. In SEC, c is negative, in LAC it is positive. In Liquid Chromatography under Critical Conditions (LCCC), c=0. If the parameters mentioned above are known with sufficient accuracy, one may predict the separation and even simulate chromatograms of very complex polymers [1,2].

The interaction parameter c and the pore surface S_p of a given repeat unit in LAC can be easily determined by a procedure published in recent papers [3,4], which also yields the the accessible volume V_0* of a column. The interaction parameter is independent on column dimensions and pore diameter and can thus be used as a measure of the interaction of a given repeat unit with the surface of a stationary phase in a given mobile phase. In LAC, there is a linear dependence between the interaction parameter and the composition of the mobile phase. Different columns with the same chemical nature fall quite well on one straight line, the intercept of which represents the interaction parameter in the poor solvent (c_A), and the slope dc/dB the strength of the good solvent.

The intersection point with the x-axis corresponds to critical conditions. Hence the interaction parameter of a given repeat unit in the LAC range can be calculated in any mobile phase composition for any column, if c_A and dc/dB are known from a data base.

The retention factor k* of a given oligomer with n repeat units depends on the interaction parameter c, the length of the repeat unit, the pore surface S_p of the stationary phase and the accessible volume V_0* of the column.

The pore surface can be determined from the elution volumes of nonfunctional oligomers [3,4]. As the interaction parameter of a given repeat unit in a given mobile phase is the same for stationary phases with the same chemical nature, retention can be adjusted by selection of the pore surface [5].

Based on the interaction parameter c, the accessible volume V_0*, and the pore surface S_p, one may calculate the elution volumes of the individual oligomers in isocratic and gradient LAC [6].

References

[1] B. Trathnigg, A. A. Gorbunov, A. M. Skvortsov, J. Chromatogr. A 890 (2000) 195-210.
[2] A. A. Gorbunov, A. V. Vakhrushev, Polymer 45 (2004) 7303-7315.
[3] B. Trathnigg, A. Skvortsov, J. Chromatogr. A 1127 (2006) 117-125.
[4] B. Trathnigg, O. Jamelnik, A. Skvortsov, J. Chromatogr. A 1128 (2006) 39-44.
[5] B. Trathnigg, O. Jamelnik, J. Chromatogr. A 1146 (2007) 78-84.
[6] B. Trathnigg, M. I. Malik, O. Jamelnik, N. V. Cuong, Anal. Chimica Acta 604 (2007) 39-44.

L-17

NANOMANIPULATION OF BIOMOLECULAR SYSTEMS WITH OPTICAL TWEEZERS

Zsolt Mártonfalvi, Pasquale Bianco, Gyula Kotek, Péter Zádori, Károly Zieber, Miklós S. Z. Kellermayer

Department of Biophysics, University of Pécs, Faculty of Medicine

Optical tweezers use light to mechanically manipulate microscopic refractile objects with piconewton forces and nanometer displacements. Photons within a focused laser beam exchange momentum with the dielectric particle, such as a latex or quartz bead, so that forces are generated which tend to push the particle towards an equilibrium position called the optical trap. The restoring optical forces contribute to a virtual spring constant, therefore optical tweezers can be used to measure the miniscule external forces acting on the trapped particle following proper calibration. In our apparatus, due to its special geometrical arrangement, direct force measurement based on detecting photon momentum change is possible. We use optical tweezers to manipulate various biomolecular systems such as DNA, chromatin, elastomeric proteins and single cells, to reveal their elasticity, force-driven structural changes and the dynamics of their interactions.

A double stranded DNA molecule behaves as an entropic, wormlike chain at low forces. When stretched with a force of ~65 pN, the molecule undergoes a cooperative structural transition that significantly extends its length. The overstretch transition is reversible and the molecule recovers when allowed to shorten. In the presence of purified histones a single chromatin strand is reconstituted. Upon stretch, a significantly shortened molecular length is detected, which is the result of DNA wrapping around nucleosome particles. Around 35 pN repetitive transitions appear, each of which extend the length by ~50 nm. The transitions correspond to the unwrapping of DNA from individual nucleosome particles. By continuing the mechanical stretch, the DNA molecule can be fully cleared of nucleosomes, and in lieu of sufficient histone concentration the chromatin strand does not recover even if the DNA molecule is allowed to shorten.

Optical tweezers are particularly optimal to explore mechanisms of force-driven protein folding mechanisms. Upon stretching the giant multimeric muscle protein titin, sawtooth-shaped transitions appear in the force trace between 30-200 pN. The transitions correspond to the unfolding of individual protein domains that extend the molecule by ~28 nm. To explore the details of both the unfolding and refolding processes, we stretch the molecule under clamped forces using force feedback. When stretched to high constant force, ~28-nm steps appear in the extension trace, directly revealing the single-domain unfolding steps. The unfolding steps follow a single-exponential fuction, the rate constant of which corresponds to the unfolding rate at the given clamp force. To explore the refolding process, a mechanically unfolded molecule is allowed to shorten at a low clamped force (1-5 pN). Titin refolds not in steps, but in three broad stages: rapid collapse, extension fluctuations, and final contraction. By using quasi force-ramp experiments we find that rapid collapse is not driven solely by chain entropy.

Finally, single living cells may be directly trapped and mechanically manipulated as well. Because our instrument does not rely on stiffness calibration, measurement of forces within the inhomogenous cellular environment is possible. In sum, optical tweezers is a special tool ideally suited to investigate molecular mechanics within a wide array of biomolecular systems.

L-18

LIQUID CHROMATOGRAPHY-MASS-SPECTROMETRY (LC-MS) FOR TRACE ANALYSIS: THE DETECTION SPECIFICITY AND ITS IMPROVEMENTS

Xinghua Guo, Martin Trötzmüller, Ernst Lankmayr

Institute of Analytical Chemistry and Radiochemistry, Graz University of Technology, Technikerstrasse 4, 8010 Graz, Austria
x.guo@tugraz.at

Nowadays the hyphenated technique liquid chromatography-mass spectrometry (LC-MS) is considered to be the method of choice for very broad applications. Compared to other means of HPLC detections, mass spectrometry offers its unique detection specificity and selectivity by analyzing / detecting analyte ions. Furthermore, the application of tandem MS (product ion, precursor ion and neutral loss scans) and selected ion monitoring (SIM) make LC-MS a very sensitive technique. However, challenges and obstacles are also encountered for trace analysis in complex matrices. This mainly due to the fact that the current interface technique atmospheric pressure ionization, such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), produce ions with very high efficiencies from not only analytes but also LC mobile phases and contaminants. The latter refers to chemical background noise and causes significant interferences in LC-MS trace analysis.
In this presentation, a general overview of the MS detection techniques in LC-MS will be given with emphasis on the past and current improvements of sensitivities. A few examples (both hardware and software approaches) to reduce background noise will be discussed, which includes our recently patented technique based on exclusive gas-phase reactions of background ions with a reactive collision gas. The improvement of the signal-to-noise ratio by up to 10 times can be achieved on our custom-modified triple quadrupole LC-MS. Some recent developments will also be discussed.

References

[1] Busch, K.: Chemical noise in mass spectrometry. *Spectroscopy* 17, 32 (2002).
[2] Guo X., Bruins A.P. and Covey T.: Characterization of typical chemical background interferences in atmospheric pressure ionization liquid chromatography-mass spectrometry, *Rapid Communications in Mass Spectrometry*, 20, 3145–3150 (2006).
[3] Guo X., Bruins A.P. and Covey T.: Method to reduce chemical background interference in atmospheric pressure ionization liquid chromatography-mass spectrometry (API LC-MS) using exclusive reactions with the chemical reagent dimethyl disulfide, *Analytical Chemistry* 79(11), 4013-4021 (2007).
[4] Guo X., Bruins A.P. and Covey T.: Chemical noise reduction in mass spectrometry, *PCT International Patent*, WO 2007/092873, 2007.

L-19

DETERMINATION OF DIFFUSION COEFFICIENTS FOR BIOSENSING APPLICATIONS

Barna Kovács, László Kiss, Damir Tesanovic, Géza Nagy

Department of General and Physical Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary,
kovacs1@gamma.ttk.pte.hu

Biosensors based on biocatalytic reactions often contain more than one sensing layers. In case of the most popular geometry of an enzyme based sensor, the signal transducer layer, (e.g. an optical sensor for small molecules) is covered with a second one, containing the enzyme. The analytical parameters of the sensor, such as the response time, recovery time and sensitivity are influenced by the layer thicknesses. The chemical reactions inside of the sensing layers, and hence, the time dependent parameters of the sensor are determined by the diffusion processes. Thick biocatalytical layer results in large signal change but the response time could be very slow. Often a tedious work should be made to find the optimal layer thicknesses. By knowing the diffusion coefficients in the layers one could use modeling computer programs to calculate approximately the optimal layer thicknesses.

One of the many different setups for the determination of diffusion coefficients is the so called "time-of-flight" method. The analyte is generated at a point-like source for an instant and a second point-like detector measures its concentration as a function of time. The diffusion coefficient could be calculated easily by knowing the time corresponding to the maximum of the curve: $D=d^2/(\tau \cdot C)$, where d is the distance between the source and the detector, τ-is the elapsed time and C is a coefficient. The precision of the results are affected by the geometry of the setup [1-4], because the C coefficient could change.

In this work experimental results obtained in solutions and gels are compared to model calculations. Differently sized and shaped electrode combinations were used in the experiments and the experimental geometry and conditions were modeled by a 3D modeling program based on the finite changes method.

The experimental results and the calculations showed that in case of microelectrodes prepared without shielding, the C value varies between 2 and 6 depending on the size of the source, and on the distance between the source and detector. Increasing electrode shielding resulted in an increase of C, in some cases values over 8 were measured and also calculated. When the generator and detector electrodes were placed in the same plane, C remained constant independently of the distances. By using the simulation a planar geometry is suggested for the determination of the diffusion coefficients in gels and polymers that also allows a relatively simple preparation and exchange of the polymeric layers. This way the diffusion coefficients of some model compound were also determined experimentally.

References

[1] B. Csóka, G. Nagy, J. of Biochem. Biophys. Meth. 61 (2004) 57.
[2] M. Mosbach, et al., Anal. Chem. 73 (2001) 2468.
[3] M.V. Mirkin, M. Arca, A.J. Bard, J. Phys. Chem. 97 (1993) 10790.
[4] M.E. Rice, et al., Neuroscience 15 (1985) 891.

L-20

ANALYSIS OF HEAT GENERATED TOXIC SUBSTANCES IN FOODS

Michael Murkovic

Institute for Food Chemistry and Technology, Technische Universität Graz (TUG)
Petersgasse 12/2; A-8010 Graz, Austria
Michael.murkovic@tugraz.at

During the recent years several groups of substances and single substances formed during the Maillard reaction have been identified in foods and their potential health risk was characterized in detail.
Heterocyclic amines are formed from amino acids and carbohydrates with (polar amines) or without creatinine (non-polar) amines. Due to the complex matrix the method of choice for the analysis is LC-MS with a tendency to MS^n techniques. The non-polar heterocyclic amines can be analyzed using fluorescence detection which is due to the very strong fluorescence quite sensitive and selective enough for no additional clean-up. The clean-up is normally done by two or three consecutive solid phase extractions.
In the year 2002 acrylamide was found in foods occurring at rather high concentrations. Due to the weak UV absorption and low molecular mass (m/z = 71) the analysis of acrylamide is critical especially if foods are strongly heated like coffee or cocoa. Acrylamide can occur in foods at rather high concentrations that in some rare cases acute toxicity might be observed. However, the main problem is the potential carcinogenicity of acrylamide which is metabolized to glycidamide which forms adducts with the DNA. The high polarity of acrylamide and the low molecular mass suggest that it is analyzed by LC-MS. A derivatization with mercaptobenzoic acid is possible. The derivatization increases the molecular weight of the analyte significantly and thereby improves the selectivity.
HMF is a substance that occurs in practically all foods and the exposure of adults is normally due to the uptake of coffee and in some cases beer. It is suggested that HMF is activated with sulfotransferases that form a highly reactive carbon that can form DNA adducts. The analysis of HMF can be done by HPLC-UV since the concentrations are rather high and the absorption wavelength of 280 nm is sufficiently selective for most foods. In some cases a derivatization with DNPH is suggested giving an even higher selectivity. In most cases no special clean-up is necessary.

L-21

CHEMICAL AND MICROBIOLOGICAL ASPECTS OF THE QUANTITATIVE ANALYSIS OF AMPHOTERICIN B

András Fittler[1], Zoltán Matus[2], Béla Kocsis[3], Lajos Botz[1]

(1) Department of Pharmaceutics and University Pharmacy
(2) Department of Biochemistry and Medical Chemistry
(3) Department of Medical Microbiology and Immunology
Medical School University of Pécs, Hungary

Amphotericin B can be determined by chemical (HPLC, spectrophotometry) and microbiological (bioassay) methods. The utilization of both during a stability test can give more detailed information about the activity and concentration change of amphotericin B solutions. Previously published HPLC methods do not lay stress on the separation of minor components present in the substance. We have observed that the bioassay conditions described in the Ph. Eur. 6. are not suitable for the measurement of the concentration change experienced during a stability test.
The aim of our study was to optimize the chemical and microbiological measurements. We have developed a HPLC method for the separation of the main heptaene and the minor tetraene components in Fungizone (Bristol-Myers Squibb). The most optimal bioassay conditions were determined where a relatively wide concentration range can be measured. With the improved methods both chemical and microbiological efficacy changes can be more accurately determined in our future stability tests.

L-22

STUDIES ON IN VITRO ANTIOXIDANT EFFECT OF SALICYLIC ACID AND ITS HYDROXYLATED METABOLITES

Pál Perjési, Mónika Kuzma, Krisztina Fodor, Zsuzsanna Rozmer

Institute of Pharmaceutical Chemistry, Faculty of Medicine, University of Pécs, Rókus str. 2., 7624 Pécs, Hungary, pal.perjesi@aok.pte.hu

Phenolic compounds are attracting considerable interest due much to their promising cytoprotective potentials. The bioactivity of phenolics may be related to their ability to chelate metal ions, inhibit the arachidonic acid cascade, and scavenge free radicals. Free radicals are supposed to be involved in development of several diseases e.g., atherosclerosis, Parkinson and Alzheimer disease, tumours, cataract etc [1].

There are several methods for characterization of antioxidant capacity of natural and synthetic antioxidants. Deoxyribose oxidation is one of a number of assays used for the detection of hydroxyl radical ($HO^{\bullet}$), the most reactive oxygen-centered radical formed under *in vitro* and *in vivo* conditions [2]. The other method used in our present work is the so called crocin bleaching test. The crocin bleaching test is *in vitro* assay for investigating alkoxyl (RO•) and peroxyl radical (ROO•) scavenger activity [3].

By means of the two tests antioxidant capacity of salicylic acid and it's and hydroxylated metabolites were investigated. In the short term tests salicylic acid proved to be an effective hydroxyl radical scavenger. Its effect, however, became prooxidant using longer incubation times. GC-MS investigation of the incubates indicated formation of hydroxylated metabolites. Hydroxylated salicylic acid derivatives –that can be formed by enzyme-catalyzed and non-enzyme catalyzed reactions - displayed remarkable antioxidant effects on both tests. Our results draw the attention of importance of redox activity of hydroxylated metabolites of xenobiotics.

References

[1] P. R. Muller: Neurological Disease. In: H. Sies, (ed): Antioxidants in disease mechanisms and therapy. Academic Press, New York, 1997. pp. 557-580.

[2] Zs. Rozmer, P. Perjési, *Acta Pharm. Hung*. **75**, (2005) 87-93.

[3] K. Fodor, A. Léhman, P. Perjési, *Acta Pharm. Hung*. **76**, (2006) 140-144.

ABSTRACTS

POSTERS

P-1 - P-82

P-01

MICROWAVE ASSISTED SYNTHESIS AND CHARACTERIZATION OF POLY (ETHYLENE GLYCOL)-β-POLY(ε-CAPROLACTONE) BY LIQUID CHROMATOGRAPHY UNDER CRITICAL CONDITIONS

Hasnat Ahmed, Bernd Trathnigg

Institute of Chemistry, Karl-Franzens University, Heinrichstrasse 28, A-8010 Graz, hasnatmalik47@yahoo.com

Amphiphilic block copolymers of polyethylene glycol monomethyl ether and ε-caprolactone (CL) were synthesised by ring opening polymerization using different catalysts (NaH, Potassium tert-butoxide and Sn.Octoate) in a one pot procedure under microwave heating. Polymerization with Sn.Octoate proceeded were rapidly for synthesis of clean products. Characterization was carried out by using liquid chromatography under critical conditions (LCCC).

P-02

HPLC METHOD FOR EXPERIMENTAL QUANTITATION OF 4-NITROPHENOL AND ITS METABOLITES FROM BILE

Attila Almási[1,2], Emil Fischer[1], Pál Perjési[2]

(1) Institute of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Szigeti út 12, 7624 Pécs, Hungary, attila.almasi@aok.pte.hu
(2) Institute of Pharmaceutical Chemistry, Faculty of Medicine, University of Pécs, Rókus u. 2, 7624 Pécs, Hungary

After the oral drug administration the molecules reach the portal blood circulation and can be metabolized in the liver. The hepatic metabolism has a main role in the elimination of the xenobiotics, the extrahepatic -including intestinal- transformations, however, may also have significant influence for the disposition of pharmacons [1].
In this work, we investigated the effect of experimental diabetes for the elimination of 4-nitrophenol, with special regard to the bile. 4-Nitrophenol (PNP) is excreted almost exclusively as its glucuronide (PNP-G) and sulfate (PNP-S) conjugates [2]. Because of this simple and well characterized metabolic profile PNP is widely used as a model substrate to evaluate the influence of drug therapy, disease, nutrient deficiencies and other physiologically altered conditions on conjugative drug metabolism in animal studies.
Hyperglycemia was induced in male Wistar rats (weighting 220-250 g) by i.v. administration of STZ in a dose of 65 mg/kg. After 1 week of STZ treatment , in case of a part of the animals a rapid-acting insulin (1 IU/kg) , in an other group of the animals slow acting insulin (2x15 IU/kg/day) were applicated before being anaesthetised with urethane (1,2 g/kg i.p.) and a jejunal loop was cannulated. We used control animals as well. The bile samples were obtained from the cannulated bile duct.
As a continuation of our investigations of intestinal perfusations [3], we developed an easy to run isocratic HPLC assay to quantitate 4-nitrophenol (PNP), 4-nitrophenyl-β-glucuronide (PNP-G) and 4-nitrophenyl-sulphate (PNP-S) in the large number of bile samples. This work describes details (focusing on within-day precision, day-to-day precision, linearity) of the optimized reversed phase HPLC assay, and its application to quantitate PNP, PNP-S and PNP-G.

References

[1] Fischer, E., Lauterbach, F., *Z. Gastroenterol*. **30** (1992) 224.
[2] Quebbemann, A. J., Anders, M. W., *J. Pharmacol. Exp. Ther*. **184**, (1973) 695-708.
[3] Almási A., Fischer E., Perjési P., J. Biochem. Biophys. Methods **69** (2006) 43-50.

P-03

INVESTIGATION OF COPPER CORROSION BY CYCLIC VOLTAMMETRY AND IMPEDANCE SPECTROSCOPY

Fouzia Altaf, Qamar Abbas, Leo Binder

Institute for Chemistry and Technology of Inorganic Materials, Graz University of Technology, Stremayrgasse 16/III, A-8010 Graz, Austria
Leo.binder@tugraz.at

Copper is different from most other metals in that it combines corrosion resistance with high electrical conductivity, formability, machineability, and strength when alloyed, except at high temperature. Copper and its alloys are widely used for the transportation of water for domestic and industrial uses. Another recent application of copper and its alloys is in area of microelectronic technology. When copper and its alloys are used in microelectronic systems, the maintenance of original surface characteristic is important and therefore formation of any corrosion product is undesirable. The introduction of copper and its alloys to the microelectronics industry requires an improved understanding of the chemistry of deposition, passivation, chelation, and corrosion.

Industrial processes are often conducted in acidic environment that are aggressive to copper. Corrosion problems often arose in acidic cleansing process although copper does not normally displace hydrogen even from acidic solution. Its corrosion rate increases with concentration of acid, amount of aeration, the presence of oxidizing agents and other chemical species.

The stability of copper against corrosion is dependent upon the chemistry, thermodynamics and kinetics of associated reactions as well as protection afford by corrosion products. The utilization of inhibitors to protect metals against corrosion is based on the ability of certain individual chemical compounds or mixtures to reduce or to completely suppress the rate of corrosion processes. Inhibitors enable reduction of corrosion rate by influencing the kinetics of corrosion processes which is consistent with the anodic and cathodic conjugate reactions.

We find from literature that for several decades the corrosion behavior of copper and effects of various organic inhibitors in acidic, neutral and alkaline solution have been explored. It was found that the corrosion rate of copper is influenced by the pH and has its lowest value in slightly alkaline solutions. When considering the papers devoted to copper corrosion and inhibition, it can be seen that investigations on corrosion of copper alloys in various mediums are very rare. The objective of this research is to perform comparative studies on the corrosion behavior of copper and its alloys over the pH range 4 to 10, to investigate adsorption behavior of azoles on copper and its alloys surface, and also to investigate effect of halide ions and their concentration on passivation behavior as well as on the corrosion of metal. Experiments were carried out with and without presence of halide ions as well as in the presence of inhibitor at room temperature and at various pH ranges by using cyclic voltammetry and impedance spectroscopy.

The degree of surface coverage of inhibitor is determined by ac impedance. Changes in the impedance parameters (charge transfer resistance (R_{ct}) and double layer capacitance (C_{dl}) are related to adsorption of organic inhibitors on metal surface. From cyclic voltammetric study derives the effiency of inhibitors.

P-04

THE ALLUVIAL DEPOSITS OF FLOOD-PLAINS OF KÖRÖS RIVER DURING THE RIVER CONTROL

Krisztina Babák

Department of Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary, babak@gamma.ttk.pte.hu

The study of the geomorphic effects of fluvial systems has always played an important role in the Hungarian geography. The habitations were threatened by the enormous hazards of flood returning year by year. The hydro-geographical environment has been altered considerably due to the flood control activities since the second half of the 1800s. This altered environment produced peculiar processes on the passive and active floodplains along the major rivers in Hungary, and diverted the natural development of the surrounding areas.

The great alluvial deposits of flood-plains weren't calculated during the works of river control in the 19th century. The fluviatile deposit transport capacity of Tisza and influents was very significant. This process was changed when the excessive deforestation began in the watershed area because of accelerated urbanization were grown during 150 years.

In my work I am dealing with measurement of alluvial deposits of flood plain. The first Hungarian taking of samples was happened by Körös River in Takács-zug. The result of examination was interesting. The alluvial deposit was 150–180 cm during the river control.

P-05

ICP-MS DETERMINATION OF REE IN DIFFERENT PARTS OF PUMPKIN (*CUCURBITA PEPO VAR. STYRIACA*) IN RELATION TO GEOGRAPHIC ORIGIN

Donata Bandoniene, Daniela Jöbstl, S. Obersriebnis, Thomas Meisel

General and Analytical Chemistry, Montanuniversität Leoben, Franz-Josef-Straße 18, 8700 Leoben, Austria, donata.bandoniene@unileoben.ac.at

Tracing the geographic origin of food has become an increasing economic factor. Customers are more and more aware of the consequences of globalization, large distance transport, fair trade etc. Chemists are challenged to provide mean on how to secure the geographic origin of products and to avoid fraud. In this respect, stable light isotope ratios have been used to identify the geographic origin of food products. There have been attempts with radiogenic isotopes (e.g. Pb, Sr, Nd etc.) and trace element the distribution patterns as potential indicator [1]. In our project, an analytical method based on determination of rare earth elements (REE) with ICP-MS for identification of the geographic origin of pumpkin seed oil using discriminate analysis was developed (see Jöbstl et al. in this volume). The REE patterns do show regional differences which could be used for tracing the geographic origin but no reason can be given for this observation. In addition, the scientific knowledge about the accumulation and distribution of REE in plants and these relations to topsoil are very sparse [2]. To understand the background for regional differences, from several geographic origins, samples were taken from the several parts of pumpkin, pumpkin leaves, pumpkin meet, pumpkin seeds, the oil extracted from the seeds (Soxhlet) and oil-extraction cake, and the topsoil. REE measurements were performed with and ICP-MS (Agilent 7500) after sample decomposition of the organic test portions in a high pressure asher (HPA-S, Anton Paar) or in a microwave (Multiwave, Anton Paar) and the soils with Na_2O_2. The important outcome of this study is that the distribution patterns of the different parts of pumpkin follow that of the continental crust, i.e., light REE enrichment, negativ Eu anomalies and more or less flat heavy REE distribution in chondrite normalized diagrams. Thus the plant does extract REE from the substrate. But the most interesting observation is that the regional variation of the REE distributions of the extracted oils is much larger than the those of the soils. Since REE are non essential elements for plants, our original hypothesis that the REE distribution patterns directly reflect that of soils substrate has been falsified. In addition we find the different accumulation of the REE in the different parts of the pumpkin. With the use of ICP-MS it is possible to obtain important information about the accumulation of REE and typical distribution patterns in various parts of pumpkin and in the soil. This can help to understand the behavior of REE in the plants and thus in food products. Hence, this study can be very useful for the application of the very promising approach based on REE patterns for identifying geographic origin of food products.

References

[1] Kelly, S., Heaton, K., Hoogewerff, J. *Trends Food Sci.Technol.* **16** (2005) 555.
[2] Bettinelli, M., Spezia, S., Baffi, C., Beone, G. M., Rocchetta, R., Nassisi, A. *At. Spectr.* **26** (2005) 41-50.

P-06

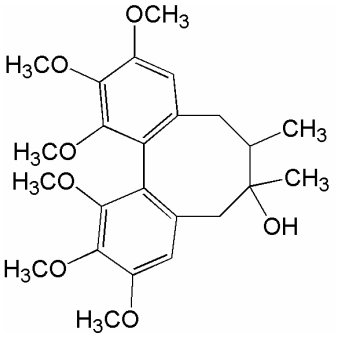
ISOLATION AND STRUCTURE ELUCIDATION OF *SCHISANDRA CHINENSIS* LIGNANS AND DERIVATIVES

Martina Blunder[1], Olaf Kunert[2], Antje Hüfner[2], Walter Fabian[3], Wolfgang Schühly[1], Rudolf Bauer[1]

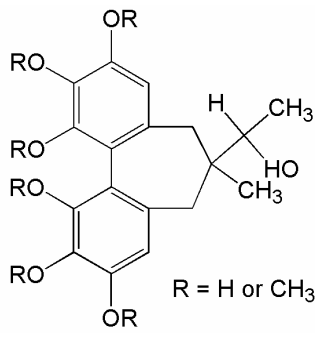
(1) Institute of Pharmaceutical Sciences, Department of Pharmacognosy,
(2) Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry,
(3) Institute of Chemistry, Department of Organic Chemistry, Karl-Franzens-University,
Universitätsplatz 4, 8010 Graz, Austria, martina.blunder@uni-graz.at

Schisandra chinensis (Turcz.) Baill. (Wu Wei Zi, Magnolia Vine, Schisandraceae) is a commonly used traditional Chinese medicinal plant possessing diverse biological activities like antihepatotoxic, antibacterial and cardiovascular effects as well as effects on CNS, uterus and immune regulatory actions. The fruits of *Schisandra chinensis* are a rich source of lignans containing a dibenzo[a,c]cyclooctene skeleton commonly with a high degree of methoxylation at the biphenyl system. Schisandrin (1), gomisin A, angeloylgomisin H, and tigloylgomisin H were isolated combining different chromatographic techniques such as LC, SPE, prep HPLC etc.

In order to obtain more polar derivatives of *Schisandra* lignans demethylation with BBr_3 under different conditions was evaluated. The complex product mixture was purified by means of semipreparative HPLC. Resulting derivatives (2) showed a (partial) demethylation and/or reduction in ring size. Structure elucidation of the natural occurring lignans as well as derivatives thereof was carried out by means of 1- and 2-dimensional NMR spectroscopy. Absolute configuration of the atropisomeric biphenyl system was determined by comparing recorded CD spectra with simulated ones (TDDFT/IEFPCM).



(1)



(2)

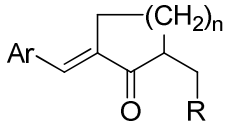
P-07

APPLICATION OF CELL-CHIP METHOD TO RAPID SUSCEPTIBILITY TESTING

Orsolya Bouquet[1], Ildikó Kustos[2,4], Tamás Lóránd[3], Ferenc Kilár[1], Béla Kocsis[4]

(1) Institue of Chemistry, Faculty of Sciences, University of Pécs, Ifjúság u. 6., 7624 Pécs, Hungary, bouquet@gamma.ttk.pte.hu
(2) Department of Microbiology, Royal Liverpool Children`s NHS Trust, Alder Hey, Liverpool, UK
(3) Department of Biochemistry and Medical Chemistry, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624 Pécs, Hungary
(4) Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624 Pécs, Hungary

In our study a fairly new, modern technology, the cell-chip method was tested to examine antifungal susceptibility. The importance of *Candida* infections has grown so as the request of rapid, easy detection of the susceptibility against antifungal agents. *Candida albicans ATCC 90028* international standard strain was used in these experiments.
The antifungal susceptibility testing was based on the differentiation between living and dead cells. Fluorescent dyes were used labelling the living and dead cells. Sytox Green non-permeable nucleic-acid dye stained only the dead cells and Syto 60 nucleic-acid dye label both dead and living cells. Based on the ratio of dead/living cells it was able to define minimal inhibitory concentrations (MIC).
Amphotericin B was applied to validate the new method.
In addition some known Mannich ketones as water soluble hydrochlorides have been tested using this technique. These substances showed according to our previous investigations antifungal effect against *Candida* strains [1].



n = 1 - 4, Ar = Ph, substituted phenyl,
R = 1-piperidyl, etc.

The commercially available Agilent 2100 Bioanalyzer and the Cell LabChip Kit were utilized.

The work was supported by the grants GVOP-3.2.1-0168, RET 008/2005 and OTKA-NKTH-NI- 68863.

References

[1] Béla Kocsis, Ildikó Kustos, Ferenc Kilár, Adrien Nyul, Péter B. Jakus, Szilárd Kerekes, László, Prókai, Victor Villarreal, Tamás Lóránd, unpublished results

P-08

ENDOGENOUS URINARY STEROIDS IN POSTMENOPAUSAL WOMEN WITH CERVICAL CANCER

Anita Bufa[1], Viktória Poór[1], Péter Gőcze[2], Ferenc Kilár[1]

(1) Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Honvéd utca 1., Pécs H-7624 Hungary
(2) Department of Obstetrics and Gynecology, Faculty of Medicine, University of Pécs, Édesanyák útja 17., Pécs H-7624 Hungary

The profile of endogenous steroids in the presence of cervical cancer was studied. Urine samples from 14 postmenopausal women with cervical cancer and 10 age-matched healthy women were collected for 24 hours. The concentration of 23 androgen, progesterone and corticoid metabolites in the urine samples of the two groups were quantitatively determined by gas chromatography-mass spectrometry with selected ion-monitoring. In the course of the urinary steroid determination we observed changes in the steroid profiles in the diseases examined compared to the same age and same sex control groups. Profiling urinary steroids has to give a comprehensive information about the synthesis of steroids including the glandular and peripheral steroid metabolisms. The concentrations of PD, PT, Δ5-AT, Δ5-PT, THB and α-C were not significantly different in the two groups. The concentrations of An, Et, 11-OH-An, 11-OH-Et, THE, THF, aTHF, α-CL and β-CL were significantly lower in the postmenopausal women with cervical cancer than in the controls. The levels of DHEA, 16-OHD, Δ5-PD, THS, 11-OPT, THA, aTHB and F could not be determined because the concentrations of these compounds were below the limit of detection (LOD) or the lower limit of quantification (LLOQ). The changes in the levels of single metabolites point out the important role of steroid groups, thus providing help in the recognition and treatment of diseased states.

The work was supported by the grants GVOP-3.2.1-0189, GVOP-3.2.1-0223, RET 008/2005 and OTKA-NKTH-NI- 68863.

Keywords: Cervical cancer; Androgens; Progesterone; Corticoids; Urinary concentration; Gas chromatography-mass spectrometry

P-09

DISTRIBUTION OF TRACE ELEMENTS, ESPECIALLY RARE ERTH ELEMENTS, IN DIFFERENT EDIBLE PLANT OILS A MEAN FOR AUTHENTICATION

Efstathios Chatzistathis[1,2], Donata Bandoniene[1], Daniela Jöbstl[1], Thomas Meisel[1]

(1) General and Analytical Chemistry, Montanuniversität Leoben, Franz-Josef-Straße 18, 8700 Leoben, Austria
(2) Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece
stathish@chem.auth.gr

Some mostly high priced edible oils such as pumpkin seed, olive, sesame seed, hazelnut, walnut, etc., used mainly for health benefits of specific fatty acids or for their flavour are always prone to adulteration [1]. The process of testing for authenticity should be approached in the different ways such as determination of fatty acid profile or minor components including sterols, tocopherols, hydrocarbons, pigments, phenols, flavouring components by HPLC, GC, GC-MS, NMR, etc. [2]. These methods are complex, expensive and time consuming. Since the concentrations of trace elements including rare earth elements (REE or lanthanides) of vegetable oils show significant oil specific differences, they are used for oil characterisation [3].

The distribution of the REE in various parts of the plants and the plant oil show significantly different distribution patterns. Furthermore, due to their low contamination potential during manufacturing process of pressed oil, this group of elements attracted our interest for the authentication of oils.

The aim of this work was to determine trace element concentrations, in particular REE, in various plant oils such as cold pressed pumpkin seed, sesame and sunflower oils, virgin olive oils and some inexpensive edible oils like refined corn, sunflower and rapeseed oils. A high sensitivity technique (ICP-MS Agilent 7500ce) was used for the exact determination of the concentrations of trace elements in oil samples completely digested with a high pressure asher (HPA-S, Anton Paar). The concentrations of REEs and the other trace elements in the refined vegetable oils tested could not be quantified in the most cases, likely because of the manufacturing process. On the other hand the trace element concentrations in cold pressed oils were found to be much higher especially for pumpkin seed oil. In addition, significant differences of REE distribution patterns of the oils were observed (t-test). In detail the concentrations of trace elements in the tested cold pressed pumpkin seed oil for Mg, Al, Ca, Mn, Fe, Cu, Ga, Rb, Sr, Ba were 11, 0.10, 25, 0.26, 2.0, 0.11, 0.0018, 0.021, 0.087, 0.061 $\mu g\ g^{-1}$, respectively. The cold pressed sunflower oil concentrations for Mg, Ca, Cr, Cu are 1.0, 3.7, 0.028, 0.016 $\mu g\ g^{-1}$, respectively. Finally the concentrations of Mg, Al, Ca, Mn, Fe, Zn, Ga, Rb, Sr, Ba in cold pressed sesame oil were determined to be 22, 0.080, 27, 0.24, 0.69, 0.85, 0.0092, 0.016, 0.32, 0.60 $\mu g\ g^{-1}$, respectively. The concentrations of traces elements in virgin olive oils in comparison with other cold pressed oils tested were found very low, likely due to removing of trace element carriers through filtering during production or due to the kind of plant thus could not be appropriately used for the detection of adulteration of olive oil.

Due to the differences between cheap refined oils and pumpkin seed oil in trace element concentrations, it was also tried to explore the possibility to detect and quantify adulteration in pumpkin seed oil with refined sunflower oil. A relatively simple and rapid analytical

procedure with ICP-MS analysis was used for the quantification of trace elements in different pumpkin seed oil mixtures with sunflower oil, ranging from 5 to 80% adulteration. As a result statistically significant differences (t-test) in the concentration of REEs and other trace elements for each mixture in pumpkin seed oil were observed and quantified. Thus, this method may allow the detection of the addition of small percentages of sunflower or other refined oil to more valuable pumpkin seed oil.

References

[1] M. Jee (2002) Oils and fats authentication, Osney Mead, Oxford OX2 0EL, UK, Backwell Publishing Ltd: 211.
[2] J. Parcerisa, D.G. Richardson, M. Rafecas, R. Codony and J. Boatella (1998) Fatty acid, tocopherol and sterol content of some hazelnut varieties (Corylus avellana L.) harvested in Oregon (USA), J Chrom A, 805: 259.
[3] T. Meisel and D. Bandoniene (2007) A pattern recognition system for identifying a vegetable oil sample, in: European Patent Office (Ed.), Netherlands.

P-10

A KINETIC EVALUATION OF MICROWAVE-ASSISTED DERIVATIZATION PROCEDURES USING HYPHENATED MASS SPECTROMETRIC TECHNIQUES

Markus Damm[1], Gerald Rechberger[2], Manfred Kollroser[3], C. Oliver Kappe[1]

(1) Christian Doppler Laboratory for Microwave Chemistry (CDLMC) and Institute of Chemistry, Karl -Franzens- University Graz, A-801 0 Graz, Austria, markus. damm@uni-graz. at
(2) Institute of Molecular Biosciences, Karl-Franzens-University Graz, A-8010 Graz, Austria
(3) Institute of Forensic Medicine, Center for Theoretical-Clinical Medicine, Medical University of Graz, Austria

The potential of microwave-assisted derivatization techniques in systematic toxicological analysis using gas chromatography coupled with mass spectrometry (GC/MS) was evaluated. Special emphasis was placed on the use of dedicated microwave reactors incorporating online temperature and pressure control. The use of such equipment allowed a detailed kinetic analysis of several microwave-assisted derivatization protocols comparing the efficiency of microwave and conventional heating methods utilizing a combination of GC/MS and liquid chromatography coupled with mass detection (LC/MS and LC/MS/MS) techniques. The kinetic analysis revealed that for standard derivatization protocols such as acetylation (exemplified for codeine and morphine), pentafluoropropionylation (for 6-monoacetylmorphine) and trimethylsilylation (for ^{9}-tetrahydrocannabinol) a reaction time of 5 min at 100 °C in a microwave reactor was sufficient to allow for complete derivatization. Control experiments using standard operating procedures (30 min at 60 °C conventional heating) indicated that the faster derivatization under microwave irradiation is a consequence of the higher reaction temperatures that can rapidly be attained in a sealed vessel and the more efficient heat transfer to the reaction mixture applying direct in core microwave dielectric heating. The results suggest that microwave derivatization procedures can significantly reduce the overall analysis time and increase sample throughput for GC/MS-based analytical methods.

P-11

DEVELOPEMENT OF A HPLC METHOD FOR NEW ANTITUMORAL DRUGS USING UV AND FLUORESCENCE DETECTION

Fabrizio Donnarumma[1], Veronika Matzi[2], Alfred Maier[3], Ralph Herwig[4], Joachim Greilberger[5], Heinz Juan, Reinhold Wintersteiger[1]

(1) Inst. of Pharmaceutical Sciences, Karl Franzens University of Graz, Universität Platz 1, 8010, Graz, Austria; fabrizio.donnarumma@uni-graz.at
(2) Dept. of Thoracic and Hyperbaric Surgery, Medical University of Graz, Austria
(3) Dept. of Urology, Medical University of Vienna, Austria
(4) Inst. of Physiological Chemistry, Medical University of Graz, Austria
(5) Dept. of Biomedical Research, Medical University of Graz, Austria

Alpha-ketoglutaric acid (KG), hydroxymethylfurfural (HMF), N-acetylseleno-L-methionine (NASeLM) and N-acetyl-L-methionine (NALM) are currently investigated in clinical studies as a novel approach in targeted cancer therapy. The exciting angiogenesis suppressor activity of KG, the ultra-selective cytostytic activity of HMF and the anti-angiogenesis effect of NASeLM supported by NALM as stability factor are used in Karal ®, a new solution for IV infusion for therapy based on metabolic induced tumor cell destruction with minimal side-effects to normal cells; a fact which necessitates the quantification of these compounds in plasma.

Therefore, the present work describes a simple and sensitive liquid chromatographic method for the contemporary detection of the target compounds through a derivatization step.

The method is based on the reaction of these compounds with dansylhydrazine, a well-known reagent with fluorescence producing and with UV absorbing properties that reacts with carbonyl and as well with carboxyl groups under catalyzation with trifluoroacetic acid (TFAA) by this providing also the needed acidic pH. Reaction conditions as time, temperature, reagent concentration and volume where thoroughly investigated separately for each compound. Extended testing of those parameters proved to be unaterable for developing a general reaction scheme, which would be capable to produce stable and sensitive detectable derivatization products for contemporary determination of all four compounds.

The formed derivatives could be separated by reversed-phase LC on a C_8-column and analyzed by UV and fluorescence detection in a single run using gradient mobile phase with acetonitrile and phosphate buffer.

Separation of KG is closely connected with the presence of the phosphate buffer as a hydrophilic component of the mobile phase in a ratio of 70:30 with acetonitrile. These conditions suppress the fluorescence quantum yield of the dansylhydrazone derivative which has to be analyzed by UV while the other three components can be separated using a gradient and detected fluorometrically. The results obtained showed good reproducibility with detection limits down to the low picogram range.

P-12

MECHANISTIC STUDY OF ENZYMATIC AND CHEMICAL HYDROLYSIS OF POLY(ETHYLENE-TEREPHTHALATE) FABRICS

Anita Eberl[1,2], Tina Brückner[2,3], Sonja Heumann[1,2], Justyna Korpecka[2], Georg M. Gübitz[1,2]

(1) Research Centre Applied Biocatalysis, Petersgasse 14, A-8010 Graz, Austria
(2) Graz University of Technology, Dept. of Environmental Biotechnology, Petersgasse 12, A-8010 Graz, Austria
(3) Niederrhein, University of Applied Sciences, Department of Textile and Clothing Technology, Webschulstraße 31, 41065 Mönchengladbach, Germany

Synthetic fibres made from poly(ethylene terephthalate) (PET) form an important part in textile industry. Commercial production of the fibres started in 1951 and account nowadays for 73 % of all synthetic fibres on the market with an annual production of 27 million tons per year. Due to their beneficial properties like high strength, low abrasion and low production costs they are besides conventional clothing used in the areas of functional sportswear, protective clothing and medical textiles. A big disadvantage of these fibres is their hydrophobicity which decreases the physiological properties of the fabrics and causes difficulties in finishing.

Conventionally, the hydrophilicity of PET fibres is improved by sodium hydroxide treatment. A rather new approach is the use of hydrolytic enzymes. Due to their size these biomolecules specifically act on the surface and bulk properties of the polymers therefore remain unchanged.

Alkaline and enzymatic hydrolyses of PET fabrics were mechanistically compared. The release of degradation products during hydrolysis was monitored using HPLC-UV-RI detection and changes in surface properties were investigated by XPS measurements and cationic dyeing. Enzyme partially adsorbed to PET fabrics during hydrolysis was completely removed by a three-step washing procedure with an additional extraction step according to XPS measurements.

Alkaline treatment as a harsh method strongly affects the fibres resulting in pitting corrosion, whereas the surface after enzymatic treatment remained unchanged. Therefore it can be concluded, that enzymatic treatment could be very useful for polyester functionalisation under mild and environmentally friendly conditions.

P-13

SPE FRACTIONATION OF PHOSPHOLIPIDS IN BACTERIAL CELL-MEMBRANE AND DETERMINATION BY HPLC-MS/MS

Alexander Fauland, Martin Trötzmüller, G. Fauler, Xinghua Guo, Ernst Lankmayr

Graz University of Technology, Institute of Analytical Chemistry and Radiochemistry, Technikerstraße 4, 8010 Graz, Austria
sausal@sbox.tugraz.at

Studies of phospholipid profiles have been considered to be a method for characterizing microorganism population. However, the structure information is lost when conventional techniques such as gas chromatography are used to analyse fatty acid after hydrolysis. Here solid phase extraction (SPE) fractionation and LC-MS have been applied to study intact phospholipids of anaerobic bacteria in biogas fermentation.
In the present study, SPE is being investigated to separate individual phospholipid classes including phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), phosphatidyl inositol (PI), cardiolipin (CA), phosphatidyl glycerol (PG), phosphatidic acid (PA) and sphingomyelin (SM).
The SPE procedure is based on a combination of one 200 mg/ 3ml aminopropyl bonded cartridge and two 200mg/3ml silica gel cartridges that enable a separation of different phospholipid classes by sequential elution. [1]
Moreover a rapid and selective one dimensional thin layer chromatography detection method has been developed to identify and semi quantify the phospholipids in several SPE fractionations.
Chloroform-methanol-25% aqueous ammonia solution was found suitable for the separation of nine phospholipids on a silica gel G plate, which is combined with the use of a molybdenum blue reagent.
The final characterisation of the phospholipid fractions are realized by reversed phase HPLC-MS/MS. [2]

References

[1] Suzuki E., Sano A., Kuriki T., Miki T., Biol. Pharm. Bull., 20(4), 299-303 (1997)
[2] Xu G., Waki H., Kon K.; Ando S., Microchemical Journal 53, 29-33 (1996)

P-14

A PHARMACOKINETIC STUDY OF GINKGO PREPARATIONS

Elisabeth Feizlmayr[1], Karin Wölkart[1], Peter Dittrich[2], Eckhard Beubler[3], Fritz Pinl[4], Andy Suter[5], Rudolf Bauer[1]

(1) Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens University Graz, 8010 Graz, Austria
(2) Institute of Pharmaceutical Sciences, Department of Pharmacology, Karl-Franzens University Graz, 8010 Graz, Austria
(3) Institute of Experimental and Clinical Pharmacology, Medical University Graz, 8010 Graz, Austria
(4) Sanatorium Hansa, 8020 Graz, Austria
(5) A. Vogel Bioforce AG, 9325 Roggwil, Switzerland

Preparations from leaves of *Ginkgo biloba* L. are among the best-selling phytopharmaceuticals worldwide. The aim of a randomized, open single-dose pharmacokinetic study with 24 volunteers was the investigation and comparison of bioavailability of three different *Ginkgo biloba* preparations (Geriaforce™ tincture, EGb 761 tablets and new, not approved ginkgo fresh plant extract tablets).
A sensitive LC-ESI-MS method with solid-phase extraction was established and used to determine the active compounds (terpene lactones) bilobalide, ginkgolide A and ginkgolide B in human plasma. Blood samples were taken 15, 30, 45, 60, 90 and 360 minutes after administration of the different Ginkgo preparations (2.73 mL Geriaforce tincture, 3 EGb 761 tablets or 4 ginkgo fresh plant extract tablets). The concentrations of terpene lactones in the administered products were in the low mg range per dose.
The resulting maximum concentrations (median) of bilobalide, ginkgolide A and B in plasma were 3.53, 3.62, 1.38 ng/mL, respectively after administration of Geriaforce™ tincture, 3.82, 2.41, 1.37 ng/mL after taking ginkgo fresh plant extract tablets and 4.73, 2.90, 1.76 ng/mL after administration of EGb 761™ tablets. The maximum plasma concentrations were attained within the first hour after administration.
The study demonstrated that new ginkgo fresh plant extract tablets are in the range of commercially available products and that different *Ginkgo biloba* preparations behave quite similar concerning their pharmacokinetics, even when different formulations are administered. A better understanding of bioavailability and pharmacokinetics of herbal medicinal products could help in designing rational dosage regimes.

P-15

NMR SPECTROSCOPIC ANALYSES OF ENZYME CATALYZED REACTIONS

K. Fesko[2], C. Reisinger[1], J. Steinreiber[2], M. Schürmann[3], H.Griengl[1], W. Schnitzhofer[4], M. Vrsanska[5], P. Biely[5], A. Cavaco-Paulo[6], G. M. Gübitz[4], H. Weber[2]

(1) Research Centre Applied Biocatalysis, Petersgasse 14, A-8010 Graz, Austria c/o Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Petersgasse 12/I A-8010 Graz; Austria.
(2) Institute of Organic Chemistry, Graz University of Technology, Stremayrgasse 16, A-8010 Graz; Austria.
(3) DSM Pharmaceutical Products, Advanced Synthesis, Catalysis and Development, P. O. Box 18, 6160 MD Geleen, The Netherlands
(4) Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, A-8010 Graz; Austria
(5) Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovak Republic
(6) Department of Textile Engineering; University of Minho, 4800 Guimares, Portugal
hansjoerg.weber@tugraz.at

NMR spectroscopy offers several advantages over traditional analytical methods for the analysis of enzymatic or biocatalytic reactions [1]. It is a non-invasive methods which means that no material is destroyed during measurement and can therefore be used for further transformations. Since almost any nucleus possesses an NMR-active isotope, a wealth of different experimental possibilities exists. For example protons can be measured in a few seconds and relatively fast reactions can easily be examined. Other nuclei like ^{13}C or ^{2}D offer unique selectivity in their measurement. Spectrometers can be easily set up to measure spectra at defined time intervals and allow the measurement of kinetic profiles of such conversions. Isotopic enrichment in well selected positions of the substrate often allows information about reaction mechanisms or energy barriers of the transformations to be extracted.
This contribution focuses on two interesting conversions:

(a) Four types of threonine aldolases with different stereospecificities were tested on the aldol synthesis of phenylserine starting from benzaldehyde and glycine under kinetic and thermodynamic control [2].
(b) Two polygalacturonases were isolated from *Sclerotium rolfsii*. NMR experiments on a model substrate revealed an inverting mechanism of carbohydrate hydrolysis for both enzymes [3].

References

[1] H. Weber, L. Brecker, *"Online NMR for Monitoring of Biocatalysed Reactions", Curr. Op. Biotechnol.* **11** (2000) *572-578.*
[2] K. Fesko, C. Reisinger, J. Steinreiber, H. Weber, M. Schürmann, H. Griengl, *"Four Types of Threonine Aldolases: Similarities and Differences in Kinetics/Thermodynamics", J. Mol. Catal. B: Enzymatic* (2008) *in press*.
[3] W. Schnitzhofer, H. Weber, M. Vrsanska, P. Biely, A. Cavacao-Paulo, G. M. Gübitz, *"Purification and Mechanistic Characterisation of Two Polygalacturonases from Sclerotium rolfsii" Enz. Microbiol. Technol.* **40** (2007) *1739-1747*.

P-16

DEVELOPMENT OF CAPILLARY-SCALE MONOLITHIC SUPPORTS FOR ON-LINE SAMPLE PRE-TREATMENT IN NANOMEDICAL RESEARCH

Christina Gatschelhofer[1], Agnes Mautner[1], Franz Reiter[1], Michael R. Buchmeiser[2], Andreas Zimmer[3], Karin Wernig[3], Thomas R. Pieber[1,4], Frank M. Sinner[1]

(1) Institute of Medical Technologies and Health Management, JOANNEUM RESEARCH, Auenbruggerplatz 20, A-8036 Graz, Austria, frank.sinner@joanneum.at
(2) Leibniz Institute for Surface Modification e. V., Permoserstr. 15, D-04318 Leipzig, Germany, and Institute of Technical Chemistry, University Leipzig, Linnéstr. 3, D-04103
(3) Department of Pharmaceutical Technology, Institute of Pharmaceutical Sciences, University of Graz, Schubertstr. 6, A-8010 Graz, Austria
(4) Department of Internal Medicine, Diabetes and Metabolism, Medical University Graz, Auenbruggerplatz 15, A-8036 Graz, Austria

Nanomedicine, the utilization of nanotechnology for medical treatment and diagnostic purposes, is a novel and promising multidisciplinary research area which requires expertise from a wide range of disciplines, including pharmacy, the material sciences and analytical chemistry. The great demands placed on analytical chemistry by nanomedical research are strongly driving advances in analytical techniques. One trend in this regard is towards the use of capillary-scale separation systems. Although analytical columns in capillary format are already well developed, there is still a lack of appropriate pre-columns for on-line solid phase extraction and pre-concentration. Furthermore, the use of miniaturized particle-based pre-columns is associated with several drawbacks, such as limited availability, low stability and insufficient inter-column reproducibility.

By overcoming the drawbacks associated with particle-based columns monolithic separation media have already become powerful tools in separation science. Monoliths can be compared to a single large particle of porous material which fills the entire column volume [1] i.e. without the inter-particulate voids typical of standard separation media. Their unique structural properties enable high flow rates at comparatively low back pressure, making them ideally suited for high-throughput separations. Their simple preparation procedure furthermore enables the preparation of chromatographic columns of any desired length and with almost no limitation in column diameter.

This contribution will describe the synthesis of capillary-scale monolithic pre-columns by ring-opening metathesis polymerization [2,3] and their use for on-line solid phase extraction and pre-concentration in the field of nanomedical research. The preparation of monoliths in various column dimensions ranging from 200 to 530 µm inner diameter using norborn-2-ene as monomer, 1,4,4a,5,8,8a-hexahydro-1,4,5,8,-*exo,endo*-dimethanonaphthalene as cross-linker, isopropanol as macroporogen, toluene as microporogen and $RuCl_2(PCy_3)_2(CHC_6H_5)$ as initiator will be described. A characterization of these pre-columns in terms of hydrodynamic properties and loading capacities will be shown. We will also discuss potential applications of monolithic pre-columns for the analysis of drugs in complex matrices such as nanoparticle suspensions or human biofluids.

References

[1] Svec, F. *J.Sep.Sci.* **2004**, *27*, 1419-30.
[2] Sinner, F. M.; Buchmeiser, M. R. *Angew.Chem.* **2000**, *112*, 1491-94.
[3] Sinner, F. M.; Buchmeiser, M. R. *Macromolecules* **2000**, *33*, 5777-86.

P-17

ANALYSIS AND CHARACTERIZATION OF NATURAL ENVIROPOLYMERS - HUMIC SUBSTANCES AND LIGNINS - USING OFF-LINE COMBINATION OF LIQUID CHROMATOGRAPHY METHODS HPLC-SEC

Róbert Góra, Havlikova Danka, Milan Hutta

Department of Analytical chemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina CH-2, 842 15 Bratislava, Slovakia, gora@fns.uniba.sk

Combined LC methods designed around RP HPLC using stepwise gradients of dimethylformamide (DMF) in buffered aqueous mobile phase and a wide-pore (30nm) octadecylsilica column had been applied to the analysis of soil, peat and air particulate humic and humic-like substances (HSs) as-well-as lignin in order to demonstrate the usefullness of the approach for their characterization even at trace concentration levels. Tandem combination of spectrophotometric (DAD) and fluorimetric detection was used to get more detailed information on chromatographic behaviour of HSs. The results showed that ten-step gradient can induce distinct features of HSs and lignins. Combination of very good DMF solvating and disaggregating properties for HSs and lignins together with wide pore RP sorbent improves surface interactions of the analytes and suppresses influence of size exclusion effects. Thus it provides reproducibility of characterization profiles and robustness of the methods. Very good reproducibility of retention times (± 0.5 % RSD), of peaks enforced by the step gradient shape supports well defined characterization and/or fractionation of HSs. Individual fractions obtained by the described RP-HPLC method were analysed by the chromatographic methods working on independent principles, e.g. SEC or IEX. SEC enables to achieve relatively precise and accurate determination of molecular mass distribution under the assumption and condition that separation or fractionation is based entirely on separation according to size of molecule. However, size of HL molecule is directly proportional not only to their relative molar mass, but is adversely influenced also by many other factors. Hence, these factors (e.g. HL concentration, pH and ionic strength of mobile phase) may have influence to their elution behaviour in a given particular separation system and overall shape of chromatographic record. Choice of a proper molecular mass calibration standards mimicking elution behaviour of HL in a given chromatographic system is very important with this respect.

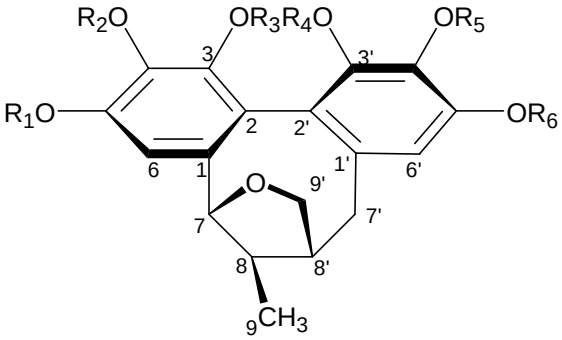
This work was supported by the financial support of projects VEGA 1/4474/07 provided by Slovak Grant Agency.

ISOLATION OF DIBENZOCYCLOOCTADIENE-TYPE LIGNANS FROM *TALAUMA GLORIENSIS*

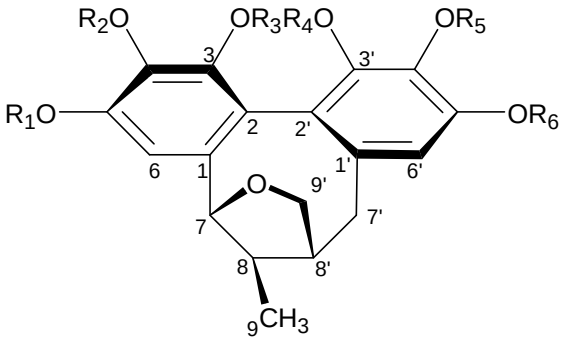
Barbara Gröblacher, Olaf Kunert, Wolfgang Schühly

Institute of Pharmaceutical Sciences,
Universitätsplatz 4[a] and 1[b] 8010 Graz, Austria
wolfgang.schuehly@uni-graz.at

In continuation of our research on the phytochemistry and pharmacology of Magnoliaceae, *Talauma gloriensis* PITTIER (= *Magnolia gloriensis* (PITTIER) GOVAERTS) collected in the low-land tropical rain forest of Costa Rica was investigated for the first time. Species of *Talauma*, e.g. *T. ovata* St.-HIL., have been described since the 19th century as medicinal plants [1]. Purification of the dichloromethane extract of the leaves yielded dibenzocyclooctadiene-type neolignans (**1** to **4**), some of which are new natural compounds. The atropisomeric structures are closely related to the pyramidatins found in the North American *M. pyramidata* BARTRAM ex PURSH (= *M. fraseri* (BARTRAM) PAMP. previously described in [2]. Structural elucidation was done using NMR, ESI-MS and circular dichroism (CD).



1
$R_1=R_2=R_5=R_6=$ -CH_2-; $R_3=R_4=CH_3$



2
$R_1=R_2=R_5=R_6=$ -CH_2-; $R_3=R_4=CH_3$

3
$R_1=R_2=$-CH_2-; $R_3=R_4=R_5=R_6=CH_3$

4
$R_1=R_2=$-CH_2-; $R_3=R_4=R_5=R_6=CH_3$

References

[1] G. Dragendorff (1898). *Die Heilpflanzen der verschiedenen Völker und Zeiten.* Reprint Werner Fritsch, München 1967.

[2] Song Q, Fronczek FR, Fischer NH. Dibenzocyclooctadiene-type lignans from *Magnolia pyramidata*. *Phytochemistry* **55** (2000) 653-661.

P-19

SYNTHETIC HYDROXYCHALCONES ACT AS MODULATORS OF MITOCHONDRIAL FUNCTIONS

Juray Guzy[1], Janka Kubálková[1], Zenóbia Chavková[1], Mária Mareková[1], Vladimíra Tomečková[1], Pál Perjési[2]

(1) Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, PJ Šafárik University, Tr. SNP 1, 040 66 Košice, Slovakia, guzyju@lf.upjs.sk
(2) Institute of Pharmaceutical Chemistry, Faculty of Medicine, University of Pécs, Rókus u. 2, 7624 Pécs, Hungary

Mitochondrial electron transport chain catalyzes a series of oxidation/reduction reactions that are driven by a proton gradient across the mitochondrial membrane. The chain is a source of constant formation of reactive oxygen species [1]. Thus influence by exogenous sources could lead to increase reactive oxygen species production and toxic affection the cell. Cytotoxic effect of α, β -unsaturated carbonyl compounds is frequently associated with the expected reactivity of the compounds with the essential thiol groups in the living organisms [2]. Recently, we have investigated cytotoxic effect of some chalcones and chalcone analogs towards various tumor cell lines [3]. In our present investigations two of selected hydroxychalcones pronounced outstanding ability to modulate mitochondrial activity. At the selected concentrations characterization of effect of the compounds on mitochondrial respiratory function was accomplished. Phosphorylation inhibitory effect of the compounds was observed with pronounced effect on stimulation mitochondrial activity and higher formation of reactive oxygen species. The compounds also produced a substrate-independent increase in oxygen consumption (respiratory burst) without affecting state 3 activity of the mitochondria. In order to clarify whether alteration of oxygen consumption is associated with change in ATP synthesis, activity of the ATPase was investigated. No one from investigated chalcones increased ATPase activity which lead to uncoupling phosphorylation without ATP synthesis.

This study was supported by VEGA grant 1/0624/08 from the Slovak Grant agency for Science.

References

[1] D. L. Boveris, A. Boveris, *Front Biosci*., **1** (2007) 1014-1023.
[2] M.L. Go, X. Wu, X.L. Liu, *Curr. Med. Chem.* **12** (2005) 483-499.
[3] Zs. Rozmer, T. Berki, P. Perjési, *Toxicol. in Vitro* **20** (2006) 1354-1362.

P-20

SEPARATION AND IDENTIFICATION OF CAROTENOIDS IN SOME MEDICINAL PLANTS' FLOWERS AND INFLORESCENCE

Györgyi Horváth[1], Péter Molnár[1], László Gy. Szabó[1], Erika Turcsi[2], József Deli[2]

(1) Department of Pharmacognosy,University of Pécs, Medical School, H-7624 Pécs, Rókus str. 2, Hungary (e-mail: georgina@gamma.ttk.pte.hu)
(2) Department of Biochemistry and Medical Chemistry, University of Pécs, Medical School, H-7624 Pécs, Szigeti str. 12, Hungary

The current research project aimed at the carotenoid analysis of total extracts of the inflorescences of Canadian goldenrod (*Solidago canadensis* L.), as well as the herb of greater celandine (*Chelidonium majus* L.) and common toadflax (*Linaria vulgaris* Mill.).
The total extracts (in Et_2O) were saponified in heterogenous phase (30% KOH/MeOH) and distributed between MeOH/H_2O (9:1) and hexane, resulting the corresponding hypohasic and epiphasic fractions [1, 2] which were analyzed separately, using HPLC and CC.
The main carotenoids of the hypophasic fraction of the extract from Canadian goldenrod (*Solidago canadensis* L.) were lutein-5,6-epoxide, (9*Z*)-lutein-5,6-epoxide (neolutein epoxide B''), flavoxanthin + chrysanthemaxanthin, (9*Z*,9'*Z*)-lutein (neolutein C; ~40%!), (9*Z*)+(9'*Z*)-lutein (neolutein B; ~22%), (13*Z*)+(13'*Z*)-lutein (neolutein A) [3-6]. In the epiphasic fraction (9*Z*,9'*Z*)-lutein, (9*Z*)+(9'*Z*)-lutein and β,β-carotene were identified as main components.
The HPLC analysis of the hypophasic fraction of the flowers of greater celandine (*Chelidonium majus* L.) resulted in violaxanthin, lutein-5,6-epoxide (~70%!), flavoxanthin+chrysanthemaxanthin, (9*Z*)-lutein-5,6-epoxide (neolutein epoxide B''), (13*Z*)+(13'*Z*)-lutein-5,6-epoxide (neolutein epoxide A). The main carotenoids of the epiphasic fraction were lutein-5,6-epoxide, lutein, (13*Z*)+(13'*Z*)-lutein-5,6-epoxide, β-cryptoxanthin, β,β-carotene [4].
The hypophasic fraction of the extract from the flowers of common toadflax (*Linaria vulgaris* Mill.) contained lutein and 3'-epilutein as major carotenoids. During the analysis of the epihasic fraction the following components were identified: lutein, 3'-epilutein, α-cryptoxanthin, (9*Z*)+(9'*Z*)-α-cryptoxanthin (neo-α-cryptoxanthin B), α-carotene (β,ε-carotene) and β,β-carotene.
The paralel use of HPLC and classic column chromatography (CC) allowed the identification of numerous minor carotenoids in all extracts and fractions. The identification of carotenoids was carried out on the basis of their chromatographic and UV/VIS spectroscopic properties (t_R, adsorption affinity; λ_{max}-values, spectral fine structure: %III/II, relative intensity of the *cis*-peak: $\%A_B/A_{II}$ and $Q = A_{max}/A_{cis\text{-peak}}$), chemical reactions [furanoid-oxide reaction = 5,6-epoxide → 5,8-epoxide rearrangement; $NaBH_4$ reduction; (*E*/*Z*)-isomerization] and co-chromatography with authentic reference samples [1-3, 7].

We thank Mrs. A. Bognár, Mrs. J. Kriszt and Mr. N. Götz for their assistance, Ms. Dr. Á. Farkas and Ms. Dr. N. Papp for collecting the plant materials. This study was supported by the grant OTKA K 60121 (Hungarian National Research Foundation).

References

[1] K. Schiedt and S. Liaaen-Jensen, in *Carotenoids Vol. 1A: Isolation and Analysis* (ed. G. Britton, S. Liaaen-Jensen and H. Pfander), pp. 81-108. Birkhäuser, Basel (1998).
[2] P. Molnár, J. Szabolcs, *Acta Chim. Acad. Sci. Hung.* **99**, 155-173 (1979).
[3] L. Zechmeister, *Cis-Trans* Isomeric Carotenoids, Vitamins A and Arylpolyenes, Springer, Wien (1962).
[4] M. Baranyai, P. Molnár, J. Szabolcs, L. Radics, M. Kajtár-Peredy *Tetrahedron* **37**, 203-207 (1981).
[5] J. Deli, P. Molnár, G. Tóth, J. Szabolcs, L. Radics, *Phytochemistry* **27**,547-549 (1988).
[6] P. Molnár, E. Ősz, G. Tóth, F. Zsila, J. Deli, *Helv. Chim. Acta* **89**, 667-674 (2006).
[7] G. Britton, in *Carotenoids Vol. 1B: Spectroscopy* (ed. G. Britton, S. Liaaen-Jensen and H. Pfander), pp. 13-62. Birkhäuser, Basel (1995).

P-21

ENANTIORESOLUTION OF TRYPTOPHAN DERIVATIVES ON CANINE SERUM ALBUMIN STATIONARY PHASES

Heike Hödl, Kurt Plöschberger, Martin G. Schmid, Gerald Gübitz

Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens-University, Universitätsplatz 1, A-8010 Graz, Austria

The present work deals with the use of canine serum albumin as chiral selector in liquid chromatography. Two different syntheses to immobilize CSA as chiral selector to silica gel were used. The two different chiral stationary phases were tested in micro-HPLC and the achieved results were compared.
Furthermore the use of CSA as chiral selector in capillary–LC was tested. Capillaries were filled with fritless monolithic phases (continuous beds; CB) including the chiral selector (CSA), which were produced by in-situ polymerisation in the capillary. For immobilization CSA was allylated and added to the monomer solution before polymerisation was started. This method of preparing capillaries with such chiral stationary phases is easy to handle, inexpensive and reproducible. Additionally, the difficult and complicated preparation of frits is prevented.

P-22

TOPOGRAPHY AND SURFACE NANOMECHANICS OF LIVING CELLS STUDIED WITH AFM

Tamás Huber, László Grama, Szabolcs Osváth, Judit Fidy, Miklós S. Z. Kellermayer

Department of Biophysics, University of Pécs, Faculty of Medicine
Department of Biophysics and Radiation Biology, Semmelweis University, Faculty of Medicine

The cytoskeleton-, membrane- and membrane skeleton-dependent cell surface processes of the living eukaryotic cells are much more dynamic than envisioned previously. Dynamically regulated protrusions from cell surfaces are ubiquitous among animal cells. Such thin membrane extensions, called membrane nanotubes, play an important role in the motion, adhesion and intercellular communication of cells. Furthermore, they may also contribute to pathological processes by providing pathways of cellular invasion and infection by viral and bacterial pathogens. Nanotubes may be either intrinsic or extrinsic. Intrinsic nanotubes are cellular extrusions caused by polymerizing actin filaments. Extrinsic or passive nanotubes, by contrast, are evoked by external pulling forces. Our fundamental aim is to understand the mechanics of nanotube formation by measuring the magnitude and time-dependence of the forces that arise during the formation and maintenance of membrane tethers.

In our experiments we pulled membrane tethers from different lines of living cells kept in monolayer cultures. Membrane tethers were generated by manipulating individual cells with atomic force microscopy (AFM). We measured the force required to pull a nanotube and characterized its viscoelastic properties. We pulled membrane tethers by pressing the AFM cantilever tip onto the cell surface and then moving it away with different velocities. During pressure application onto the cell surface, we observed stress relaxation. Relaxation of force could be well fitted with a double-exponential function. We hypothesize that the fast component corresponds to viscous response of the lipid membrane components, and the slow process to structural rearrangements within the membrane skeleton.

In the force data observed during tether stretch we observed plateaus and steps. The force plateaus describe the elongation of nanotubes at constant pulling force, while the force steps correspond to the sequential detachment of individual tethers from the cantilever. The plateau-force histogram shows multimodal distribution which gives the mean value of the tether force, 35 pN.

By studying a tumorous cell line (pancreas carcinoma, PANC) transfected with YFP-cytokeratin, and using a synchronized total internal reflection fluorescence (TIRF) and AFM system, we compared the nanomechanical behavior of membrane tethers pulled from the cell periphery and the cell center. Our observations indicate that there are no major differences in the nanomechanics of passive membrane nanotube behavior across the cell surface.

P-23

ANALYSIS OF WINE AND GRAPE SAMPLES WITH MALDI-TOF-MS

Violeta Ivanova[1,2], Ágnes Dörnyei[3], László Márk[4], Ferenc Kilár[3,5], Marina Stefova[1], Trajće Stafilov[1], Borimir Vojnoski[2]

(1) Institute of Chemistry, Faculty of Natural Sciences and Mathematics, University of Skopje, Arhimedova 5, 1000 Skopje, Republic of Macedonia
(2) Wine Department, Institute of Agriculture, University of Skopje, Blvd Aleksandar Makedonski bb, 1000 Skopje, Republic of Macedonia
(3) Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7624 Pécs, Hungary
(4) Department of Biochemistry and Medical Chemistry, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624 Pécs, Hungary
(5) Institute of Bioanalysis, Faculty of Medicine,University of Pécs, Szigeti út 12., 7624 Pécs, Hungary
dornyei@gamma.ttk.pte.hu

A rapid MALDI-TOF-MS method introducing a new matrix, fullerene [C_{70}], has been developed for fingerprinting and for analyzing the anthocyanin content of wine and grape samples. Anthocyanins are red pigments, localized in the skin of the grapes, in the first external layers of the hypodermal tissue and exclusively in the vacuoles. They are based on five anthocyanins (cyanidin, peonidin, petunidin, delphinidin and malvidin). Those compounds are present in aglycone forms in the grapes skin and glycoside forms in the wines. Malvidin-3-glycoside is the main component responsible for the red color of the wine. The wine and grape samples have been analyzed without preparation. Different MALDI matrices have been tested: α-cyano-4-hydroxycinnamic acid, 2,5-dihydroxy-benzoic acid, sinapic acid, fullerene [C_{70}] and measurements of some samples without matrix have been done. It was found that fullerene C_{70} gave satisfactory results for identification of the anthocyanins. Sandwich method has been applied for measurements of grapes and wines from Vranec, Merlot, Chardonnay and Smederevka varieties (harvest 2007). The wine samples have been obtained by different technological wine-making techniques in order to check the effect of two doses of SO_2, two yeasts (*Saccharomyces cerevisiae*, the most commonly used) from different manufactures (Macedonian yeast-Vinalko and French yeast-Levuline CHP) and the maceration time of 3, 6 and 10 days (only for red wines).

The work was supported by the grants GVOP-3.2.1-0168, RET 008/2005 and OTKA-NKTH-NI- 68863, and Ceepus HU-010 Network scholarship.

P-24

HIGH-THROUGHPUT MASS SPECTROMETRIC ANALYSIS OF NATURAL STEROIDS

Éva Jámbor, Zoltán Patonai, Alexandra Váczy, László Márk

University of Pécs, Medical School, Institute of Biochemistry and Medical Chemistry, H-7624 Pécs, Szigeti str. 12.

Establishing identity from human skeletal remains is of vital importance in the field of military exhumation,forensic osteology, physical anthropology and bioarchaeology. With the classical methods is impossible the gender determination of fragmented and infantile bones. Sexual hormones and other steroids are essential biomolecules in human and animal organisms with pronounced biological activities at low concentrations.

A rapid high-troughput, sensitive matrix assisted laser desorption/ionization time of flight mass spectrometric (MALDI TOF MS) technique has been developed for the analysis of steroids in human tissues. The method was used for molecular sex determination of ancient and forensic human skeletal remains and it was thoroughly tested with well known clinical and forensic human bone samples. The underivatised steroid hormones as estrone, estradiol, estriol, progesterone, and testosterone were analyzed by using C_{70} fullerene as matrix material at the first time.

We succesfully extracted and detected from recent forensic remains as well as 7000-year-old archaeological bone samples and used the method for gender determination of these human bones. Our results show the hormone mass fingerprinting (HMF) method is extremely suitable for rapid sex determination of fragmented forensic and paleoanthropological remains.

P-25

SALIVARY PROTEOMICS OF CLEFT PALATE PATIENTS BY USING MALDI-TOF-MS

Éva Jámbor, Réka Tihanyi, Tibor Gyula Szabó, Gyula Szabó, László Márk

University of Pécs, Medical School, Institute of Biochemistry and Medical Chemistry, H-7624 Pécs, Szigeti str. 12.

Cleft palate is birth defect that happen while a baby is developing in the uterus. During the 6th to 10th week of pregnancy, the bones and tissues that form the roof of the mouth (hard palate) or the soft tissue in the back of the mouth (soft palate) and the upper lip, which don't grow normally together. Treatment usually begins in the first few month of an infant's life. Children with oral clefting are controlled by a cleft palate team specialists through young adulthood. Complex treatment is needed and care can be lifelong.

In this study matrix-assisted laser desorption ionization tandem time-of-flight (MALDI TOF/TOF) mass spectrometry was used as a high-throughput analytical technique for identification of cleft palate stimulated proteins at the first time. Reversed phase high performance liquid chromatography (RP-HPLC) coupled by electrospray ionization ion trap mass spectrometry (ESI IT MS/MS) was used for confirmation of the peptide mass fingerprint (PMF) results.

The presence of cleft palate stimulated the expression of several proteins, included novel keratinocyte secreted proteins (e.g. Dermokine), complement factor I and other potential biomarkers.

P-26

ANALYSIS OF ORAL TUMOR REGULATED SALIVARY PROTEINS

Éva Jámbor, Alexandra Váczy, Viktória Németh, László Márk

University of Pécs, Medical School, Institute of Biochemistry and Medical Chemistry, H-7624 Pécs, Szigeti str. 12.

Oral cavity cancer is a disease in which malignant cells form in the mouth. It includes the front two thirds of the tongue, the gingiva, the buccal mucosa, the bottom of the mouth under the tongue and the hard palate. Tobacco and alcohol use can affect the risk of developing cancer.

The detection of oral tumor at an early stage is the most effective means to improve survival and reduce morbidity. One factor behind oral cancer's high mortality is the challenge detecting it with use of saliva, which has been a major meaning that has yet to come to fruition. Comprehensive analysis and identification of the proteomic content in human saliva is a necessary first step toward the discovery of saliva protein markers for human disease detection.

The salivary proteins were separated by using 1D-SDS-PAGE, and tryptic peptides of the protein were identified by matrix-assisted laser desorption ionization tandem time-of-flight (MALDI TOF/TOF) mass spectrometry. Significant differences were detected on SDS gel between the normal control and the pathological samples. Salivary proteins were identified by using Mascot peptide mass fingerprint (PMF) and MS/MS database search engine. Our results show the Annexin I, the Annexin II and several tumor related proteins were significantly up-regulated in the pathological samples.

Non-invasive salivary diagnostics promise an exciting new way to diagnose many diseases in their earliest, most treatable stages.

P-27

IDENTIFICATION OF THE GEOGRAPHIC ORIGIN OF PUMPKIN SEED OIL

Daniela Jöbstl[1], Donata Bandoniene[1], Thomas Meisel[1], Efstatios Chatzistathis[1,2]

(1) General and Analytical Chemistry, Montanuniversität Leoben, Franz-Josef-Straße 18, 8700 Leoben, Austria, daniela.joebstl@unileoen.ac.at
(2) Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, GR- 54124 Thessaloniki, Greece

In the age of the global trade and the climate changes and global warming the geographic origin of food became a factor of importance. Also the desire of the consumer for food of known geographic origin has increased, hence it is possible to buy food in supermarkets with a declaration of geographical origin at a higher price than such without traceable origin.
The aim of this work is, to develop an analytical method for the control of the geographic origin of pumpkin seed oil. The development of such a method is not only of interest for scientists but also of importance for the consumer wanting to know the origin of the food products and the assurance of the purity and quality.
It is known that the group of rare earth elements (REE the *4f* elements also called lanthanoids) in plants also have a characteristic distribution pattern similar to geological samples [1]. In addition, pumpkin seed oils of different geographic origin show variable trace element and rare earth patterns, therefore it is possible to trace the origin of these oils.
Since the REE concentrations are extremely low in pumpkin seed oil a fast and sensitive analytical method with ICP-MS had to be developed and validated. In the current project pumpkin seed from different regions in Austria and from abroad were sampled. The trace element patterns in the extracted oil of these seeds were determined and a preliminary classification with discriminate analysis was successfully done on a statistic basis. [2, 3, 4].

References:

[1] B. Markert; VCH Verlagsgesellschaft mbH, Weinheim(1993) 265
[2] C. Benincasa, J. Lewis, E. Perri, G. Sindona, A. Tagarelli; Anal. Chim. Acta **585** (2007) 366-370
[3] A. Heier; Dissertation Berlin (2006)
[4] G. Thiel, G. Geisler, I. Blechschmidt, K. Danzer; Anal. Bioanal. Chem. **378** (2004) 1630-1636

P-28

BETA-AMYLOID-BASED NANO-NETWORKS EXPLORED WITH ATOMIC FORCE MICROSCOPY

Árpád Karsai, Mátyás Kolsofszki, Ünige Murvai, Katalin Soós, Botond Penke, Miklós S.Z. Kellermayer

Department of Biophysics, University of Pécs, Faculty of Medicine

Amyloid fibrils are present in the extracellular space of various tissues in neurodegenerative and protein misfolding diseases. They may have nanotechnology applications due to their self-assembly properties and stability, provided that they can be chemically addressed and their growth and orientation controlled.

In the present work we analysed amyloid ß25-35 (Aß25-35), a toxic fragment of Alzheimer's beta peptide by using atomic force microscopy. The wild-type Aß25-35_wt formed trigonally oriented branched network on mica surface. Oriented binding depended on the cooperative interaction of a positively-charged moiety on the Aß25-35 peptide with the K^+-binding pocket of the mica lattice. By varying K^+ concentration the growth rate and the mesh size of the oriented amyloid fibril network could be tuned. In principle, beta-sheet-breaker (BSB) peptides might also alter fibril growth and stability. Our preliminary measurements indicate that the BSB peptide LPFFD, when added to a mature Aß25-35 network, does not significantly affect amyloid fibril network structure.

To add chemical reactivity, we used a mutant peptide, Aß25-35_N27C. We found that Aß25-35_N27C forms epitaxially growing fibrils on mica which evolve into a trigonally oriented branched network. Binding and fibril growth are more sensitive to cation concentration than those of the wild-type peptide. By nanomanipulating Aß25-35_N27C fibrils with a gold-coated AFM tip we showed that the sulfhydryl group of Cys27 is reactive and accessible from the solution.

We investigated thermally-induced changes in the morphology of the oriented Aß25-35_wt fibril network. The fibrils maintained a high orientation stability in the 30-70 °C temperature range, suggesting that orientational rearrangement of Aß25-35 fibrils on mica is an unfavorable process. Above 45 °C a gradual decrease in fibril length and dissociation from the surface were observed.

In sum, the oriented network of Aß25-35_N27C fibrils may be specifically labeled and utilized for constructing nanobiotechnological devices.

P-29

COMPATIBILITY OF DRUGS IN AN IMPLANTABLE INFUSION SYSTEM

Roman Keimel[1], Anton Sadjak[2], R. Likar[3], Nina Gruber[1], Astrid Sottler[1], Reinhold Wintersteiger[1]

(1) Institute of Pharmaceutical Sciences, Karl Franzens University of Graz, Austria; roman.keimel@uni-graz.at
(2) Institute of Pathophysiology und Immunology, Medical University of Graz, Austria
(3) Department of Anaesthesiology and Intensive Care, General Hospital Klagenfurth, Austria

Studies were performed with a pump designed as a flow regulated implantable pump having a reservoir of 30 ml for the continuous long term application of suffentanyl, fentanyl, normal and low-molecular mass heparin, morphine, bupivacaine, clonidine, baclofen and ropivacaine in out-patients. The device consists of a gas driven two-chamber system containing the drug solution in one chamber and the propellant in the other. At body temperature the propellant underlies an isobaric expansion, thus creating pressure towards the impermeable, flexible membrane that separates the two chambers. Subsequently the pharmaceutical preparation is transported via a catheter to the target organ. After filling the pump with drug solutions the stability of these solutions and their compatibilities with the pump materials were examined. The solutions were incubated at 37 °C for a period of 56 days. The content of the drugs as well as possible degradation products were determined by HPLC using latest versions of Pharmacopoeias recommended techniques after appropiate adaptions. For the stability tests of normal heparin and low-molecular mass heparin the official USP 28 method was used. With the exception of suffentanyl and partly fentanyl good stability in all other cases is given for the medication pump.

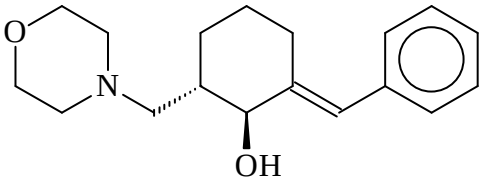
P-30

ANALYSIS OF THE VIBRATIONAL SPECTRA OF SOME ANTIBACTERIAL AMINOALCOHOLS

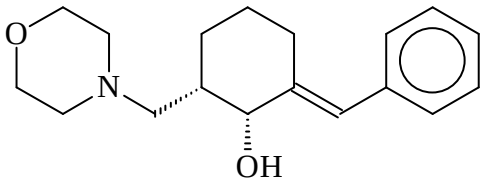
Gábor Keresztúry[1], Tom Sundius[2], Tamás Lóránd[3]

(1)Chemical Research Center, Hungarian Academy of Scienses, P.O. Box 17, H-1525 Budapest, Hungary, kergabor@chemres.hu
(2)Department of Physical Sciences, University of Helsinki, P.O. Box 64, FIN-00014 Helsinki, Finland tom.sundius@helsinki.fi
(3)Department of Biochemistry and Medical Chemistry, University Medical School of Pécs, Szigeti út 12., 7624 Pécs, Hungary, tamas.lorand@aok.pte.hu

Previously we have studied the reduction of cyclic Mannich ketones having antibacterial activity. The reduction with sodium borohydride often yielded a single stereoisomer. The size of the ring strongly influenced the stereocomposition of the reaction mixtures. An increased preference for the *trans* isomer was attributed to a weak intramolecular hydrogen bond between the OH and the N, as it was demonstrated by X-ray crystallography.
In the case of the six membered Mannich ketones the reduction afforded both the cis and the trans diastereoisomer (***cis*-1** and ***tr*-1**). We wished to investigate these isomeric aminoalcohols using the vibrational spectroscopy in order to explore the intramolecular hydrogen bond at compound ***tr*-1**.



***tr*-1**



***cis*-1**

The vibrational spectroscopic study consists of measurement of FT-IR and Raman spectra of the reaction products and subsequent DFT quantum mechanical calculations (prediction) of the vibrational spectra for the anticipated structural varieties of the synthesized molecules. Comparison of the measured and computed frequencies as well as the observed and simulated spectra is performed to resolve any uncertainties in identifying the structure of the reaction products.

References

[1] Lóránd, T., Ősz, E., Kispál, Gy., Nagy, G., Weckert, E., Luebbert, D., Meents, A., Kocsis, B., Prókai, L., *Arkivoc* vii, (2004) 34-52.

P-31

COMPARISON OF THE MASS TRANSFER KINETICS IN TOTALLY POROUS, SUPERFICIALLY POROUS AND NONPOROUS REVERSED PHASE IN LIQUID CHROMATOGRAPHY

Ibolya Kiss, Ivett Bacskay, Attila Felinger

Department of Analytical and Environmental Chemistry, Faculty of Science, University of Pécs, Ifjúság útja 6. H-7624 Pécs, Hungary

The mass transfer resistances in a stationary phase particle have several sources: the axial dispersion in the stream of mobile phase (D_L) and the external mass transfer resistance (k_{ext}); intraparticle diffusion (D_p); and kinetics of adsorption-desorption [1].
In a porous particle, solutes transfer from moving mobile phase into the stagnant mobile phase within the pores to interact with the stationary phase, and then solute molecules must diffuse out of the particle and continue its journey down the column. Such a transfer occurs as the differential separation process proceeds and the solute is eluted from the column. Porous stationary phase is universally used is HPLC for analytical and preparative purposes.
Non-porous reversed-phase packing is extensively employed forthe separation of monomeric and polymeric bioactive compounds [2]. The use of nonporous supports leads to fast kinetics. Unfortunately, the thin layer of stationary phase limits the capacity of the packing, making it unsuitable for preparative separations.
Superficially porous packing offers an attractive combination of properties. Its surface area is larger resulting in lower pressure drop and increased sample capacity. That kind of stationary phase is recommended for larger biomolecules [3].
In this study three columns were investigated: a fully porous Symmetry C18, a nonporous Kovasil-H and a superficially porous Halo C18. We are going to present the mass transfer kinetics of insulin depending on the support material geometry.

The work was supported by the grants GVOP-3.2.1-0168, RET 008/2005 and OTKA-NKTH-NI- 68863.

References

[1] Guiochon, G., Felinger, A., Shirazi, D., Katti, A. M., 2006 Fundamentals of Preparative and Nonlinear Chromatography. Academic Press, Boston
[2] Ohmacht, R., Boros, B., Kiss, I., Chromatographia 50 (1999) 75-81
[3] Cavazzini, A., Gritti, F., Kaczmarski, K., Marchetti, N., Guiochon, G., Analytical Chemistry 79 (2007) 5972-5979

P-32

DETERMINATION OF DIFFUSION COEFFICIENTS IN GELS WITH PLANAR ELECTRODE ARRANGEMENT

László Kiss[1], Damir Tesanovic[2], Géza Nagy[1], Barna Kovács[1]

[1]Department of General and Physical Chemistry, University of Pécs, , Ifjúság str. 6, Pécs 7624
[2]South-Transdanubian Cooperative Research Centre, Ifjúság str.6, Pécs 7624
kovacs1@gamma.ttk.pte.hu

The diffusion is the only mass transport process present in the biocatalytic layer of chemical sensors. By knowing it one could make estimation on the optimal layer thickness. Unfortunately, the determination of the diffusion coefficient in plasticized polymers or gels is not easy. Therefore just few compounds and polymer combination were already tested.
The measurements are based in many cases on the so called time-of-flight method. The compound of interest is emitted from a point-like source for an instant and a second point-like detector measures its concentration as a function of time. If the time corresponding to the maximum of the signal change curve, and the source-to-detector distance are known, the diffusion coefficient could be calculated easily by using the Stokes-Einstein equation.
Two different source types are commonly used. One is the electrochemical generation of a compound at the surface of a microelectrode. A more general method is the so called pressure ejection method, where the analyte is emitted from a micrometer sized capillary by applying pressure on it for some milliseconds. The precision of the time-of-flight measurements could be enhanced by repeating the measurements several times at different source-to-detector distances.
In this work the precision of the determination of diffusion coefficients were tested at two source-detector geometries. By using a fixed electrochemical source, the detector's position was adjusted with a scanning electrochemical microscope (SECM). In this arrangement the common 200-300 μm thick glass shielding of the electrodes were removed and replaced by a thin (0.5-0.8 μm) insulating layer. The source-to-detector distance was varied in the 100-800 μm range with the SECM precisely. This way the effect of the source's diameter on the time of flight results was measured.
The major problem of the use of SECM in gels or polymers is that the structure of the matrix could be changed by moving the detector tip. The tortuosity, (and/or) porosity of the polymer or gel is changed by pushing the detector tip in the source direction, since the structure is pressed together, while holes are formed by pulling them away. Therefore in the second part of our experiments a set of Pt microelectrodes (25 μm dia) were used in planar arrangement. They were glued close to each other into a plexi-glass support, polished and the distances were measured by a microscope. One of them was set as a generator electrode (source) and the other as detectors having different distances to the source.
The experimental results obtained by the two different geometrical setups are compared and discussed in this work. The planar electrode arrangement had a further advantage, namely it was easy to make polymeric or gel layers with reproducible thickness on it. Measurements in solution and in agar-agar gel were performed and were compared by using the planar electrode cell.

P-33

CHEMOENZYMATIC MODIFICATIONS OF POLYMERS IN ORGANIC ENVIRONMENT

Justyna Korpecka[1], Sonja Heumann[2], Anita Eberl[2], Georg M. Gübitz[1]

(1) Graz University of Technology, Institute of Environmental Biotechnology, Petersgasse12, Graz, Austria,
(2) Research Centre on Applied Biocatalysis, Graz, Austria
j.korpecka@tugraz.at

Polyester materials, like poly(ethylene terephthalate) (PET) have many beneficial properties. However, due to low hydrophilicity and reactivity PET materials are difficult to functionalize under mild conditions. Enzymes, especially lipases, cutinases and esterases, have been shown to modify the surface of synthetic polymers. It is an environmentally friendly alternative to drastic chemical processes for surface functionalisation of synthetic polymers.

Conducting enzymatic conversions in organic solvents as opposed to water gives also a lot of advantages, mainly the ability to carry out new reactions impossible in water due to kinetic or thermodynamic restrictions. Therefore, the topic of the work was enzymatic introduction of anchor groups into synthetic materials (PET) by transesterification. It is a new approach and allows covalent functionalization and/or grafting with biomolecules. In this study oligomers and polymers of the linear aromatic polyester PET were treated with diverse polyesterases from, *Thermobifida fusca* and *Fusarium solani* and lipases from *Candida Antarctica* and *Thermomyces lanuginosus.* The reaction was carried out in organic environment using alcohols as a substrate. Based on the detection of the transesterification products (butylbenzoate, dibutyl terephthalate) with HPLC-UVD, the lipases were found to release the highest amounts of transesterification products of the PET materials. Changes on the polymer surface caused by the reaction were investigated with gas chromatography based on consumption of the alcohol during the reaction.

P-34

RAPID AND SIMPLE METHOD FOR EXTRACTION AND DETECTION OF GAMMA-BUTYROLACTONE

Anikó Kőnigné Péter, Tímea Dergez, Ferenc Kilár

Institute of Bioanalysis, Faculty of Medicine, University of Pécs
Szigeti Út 12., H-7624
Pécs Hungary

Gamma-hydroxybutiric acid (GHB) and their precursor gamma-butyrolactone (GBL) are components of normal mammalian metabolism, but GHB has been gaining popularity amongst club-goers as a recreational drug (liquid extasy), it can produce feelings of euphoria and to enhance sexuality. GBL has hypotethical effect in the fungi metabolism, by filamentous fungi as a signaling molecule.
The characterisation and quantification of chemical substansces in a wide variety of matrices is difficult. Many forensic samples are complex mixtures such as blood, plasma, urine and culture media.
In this study, we developed a responsible and reproductable method to detect GHB from biosamples by GC/MS and simple and rapid pretreatment before the measurements. Colorimetry assay can be used for identification of GBL with spectrophotometry.

The work was supported by the grants GVOP-3.2.1-0189, RET 008/2005 and OTKA-NKTH-NI- 68863.

P-35

ACIDITY OF SOME NATURAL AND SYNTHETIC CHALCONES

Ivan Kron[1], Pál Perjési[2], Juray Guzy[1]

(1) Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, PJ Šafárik University, Tr. SNP 1, 040 66 Košice, Slovakia, kronivan@upjs.sk
(2) Institute of Pharmaceutical Chemistry, Faculty of Medicine, University of Pécs, Rókus u. 2, 7624 Pécs, Hungary

Chalcones are intermediary compounds of the biosynthetic pathway of a very large and widespread group of plant constituents known collectively as flavonoids [1]. Flavonoids are present in fruits, vegetables and beverages from plants (tea, red wine, beer), and in many dietary supplements or herbal remedies. They have been described as health-promoting, disease-preventing dietary supplements, and have activity as cancer preventing agents [2]. Chalcones (1,3-diphenyl-2-propenones) and their analogues are of interest from both chemical and biological points of view [3]. Their possible action in human organism is given through their solubility in physiological fluids which is directly related to their acidity.
pK_a of several natural and synthetic chalcones (hydroxy- and dimethylamino-derivatives) were determined by a modified spectrophotometric method. The results were compared with the gas phase O–H bond dissociation enthalpies (D(PhO–H)) calculated by computational method. Determined pK_a and calculated D(PhO–H) values agreed well in accordance with the electron transfer within chalcone molecules which is supported also with the observations at NMR measurements with synthetic chalcones [4]. Financial support of the Slovak Grant Agency 1/2267/05.

References

[1] T.F. Stuessy, D.J. Crawford, *Plant Systematics Evol*. **143** (1983) 83-107.
[2] Y.J. Moon, X. Wang, M.E. Morris, *Toxicol. in Vitro* **20** (2006) 187-210.
[3] D.N. Dhar, The chemistry of chalcones and related compounds, Wiley, New York, 1981.
[4] P. Perjési, J. Linnanto, E. Kolehmainen, E. Ősz, E. Virtanen, *J. Mol. Struct*. **740** (2005) 81-89.

P-36

ACTIVATION OF OXIDATIVE STRESS RESPONSE BY HYDROXYCHALCONES IN MITOCHONDRIA

Janka Kubálková[1], Juray Guzy[1], Zenóbia Chavková[1], Mária Mareková[1], Vladimíra Tomečková[1], Pál Perjési[2]

(1) Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, PJ Šafárik University, Tr. SNP 1, 040 66 Košice, Slovakia, janka.kubalkova@upjs.sk
(2) Institute of Pharmaceutical Chemistry, Faculty of Medicine, University of Pécs, Rókus u. 2, 7624 Pécs, Hungary

In vitro study demonstrated assessment of adaptive mitochondrial response to treatment with four selected 4-hydroxychalcone derivatives. Oxygen derived species normaly produced by aerobic metabolism or resulted from interaction with various compounds attaching lipids, proteins and nucleic acids could cause detrimental biological consequences. Several chalcones and their synthetic analogues has been found to display dual - cytotoxic and on the other hand protective - activities. It is presumed that the toxic effects of the compounds might be due to pro-oxidant activities [1]. Cytotoxic effect of compounds are frequently associated with the expected reactivity of the compounds with the essential thiol groups in the living organisms [2]. Determination of reduced glutathione levels in mitochondria and glutathione related enzymes exposed to the tested compounds was performed. Observed increase in glutathione peroxidase activities were considered as a result of reactive oxygen species production followed by clear reduced glutathione depletion. Levels of glutathione reductase in concomitant decrease with reduced glutathione concentration were found.

This study was supported by VEGA grant 1/0624/08 from the Slovak Grant agency for Science.

References

[1] M.L. Go, X. Wu, X.L. Liu, *Curr. Med. Chem.* **12** (2005) 483-499.
[2] Zs. Rozmer, T. Berki, P. Perjési, *Toxicol. in Vitro* **20** (2006) 1354-1362.

P-37

COMPOSITION AND STABILITY OF SOLVATION SHELL OF PHENOL DERIVATIVES IN BINARY MIXTURE OF WATER AND ETHANOL. A THEORETICAL STUDY

Sándor Kunsági-Máté[1], Koichi Iwata[2]

(1) Department of General and Physical Chemistry, University of Pécs, Ifjúság 6, H-7624 Pécs, Hungary, kunsagi@gamma.ttk.pte.hu
(2) Research Centre for Spectrochemistry, University of Tokyo, Bonkyo-Ku,113-0033 Tokyo, Japan

Weak interactions between molecules possessing aromatic moieties play known determinant role in the nature. Formation of complexes in solutions assumes decomposition of the solvation shell of the interacting molecules prior their association and formation of the solvation shell around the newly formed complexes. The solvation shell of aromatic solute formed in binary mixtures has specifically complex structure [1]. In this work the composition and stability of the solvation shell of *para*-substituted (NO_2-, Cl-, H-, CH_3-, and *t*Bu-) phenol derivatives as model compounds were examined in water-ethanol binary solutions. To this study DFT/6-31++G(p,d) quantum-chemical calculations were performed and the solvent effect was considered with the Polarizable Continuum Method (PCM). The Gibbs free energy gained by the formation of the solvation shell was calculated by analysis of partition functions associated to the vibration and rotation of the interacted particles. The dynamics of the solvation shell was examined by molecular dynamics (MD) calculations using AM1 semiempirical Hamiltonian with TIP3P method. MD simulations revealed that increasing the molar fraction of ethanol in the aqueous solutions to above the 0.1 the molar fraction of ethanol molecules in the solvation shell changes unexpectedly to about 0.5 and remains unchanged while the molar fraction of ethanol in the bulk solution varies from 0.1 to 0.9. The changes obtained in the thermodynamic parameters show that the formation of binary solvation shell in the present particular case forced by the entropy gained during the shell-formation reaction. Analysis of the entropy term itself suggests the following molecular background: the rotation freedom of a water molecule presented in the solution reduces drastically when this molecule enters into the solvation shell. In contrast, the rotation of a larger ethanol molecule is lower already prior the association, therefore, its rotational freedom shows smaller reduction during the ethanol molecule enters into the solvation shell. As a result, the entropy content of the system increases during the water to alcohol interchange reaction in the solvation shell. This property slightly affected by the permittivity of the aqueous ethanol solutions. Since the role of hydrogen bonds was found to be very important in determination of the structure of the solvation shell, further investigation planned to clarify the situation in nonprotic solvents.

This work was supported by the Japan Society for the Promotion of Science (JSPS).

References

[1] Kunsági-Máté, S., Ortmann, E., Kollár, L., Nikfardjam, M.P.: *J. Physical Chemistry B* **111** (2007) 11750-11755.

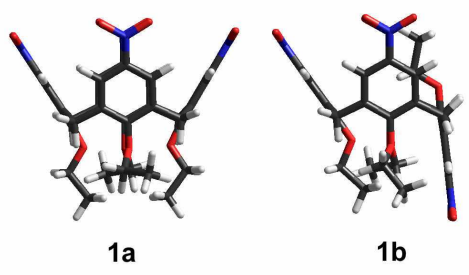
P-38

PERMITTIVITY-DEPENDENT COMPLEXATION ABILITY OF TETRANITRO-CALIX[4]ARENES TOWARDS PARA-SUBSTITUTED PHENOLS

Sándor Kunsági-Máté[1], Zsolt Csók[2], László Kollár[3]

(1) Department of General and Physical Chemistry, (3) Department of Inorganic Chemistry, University of Pécs, Ifjúság 6, H-7624 Pécs, Hungary,
E-mail: kunsagi@gamma.ttk.pte.hu,
(2) Departamento de Chímica da Universidade da Madeira, 9000-390 Funchal, Madeira, Portugal

Considering the importance of the polarizability of the rings of calixarenes in the entropy-driven complexation processes, we examined the effect of entropy compensation on the complex formation of *cone* (**1a**) and *partial cone (‘paco’)* (**1b**) conformers of tetranitro-calix[4]arene, possessing *O*-ethyl substitutens at the lower rim, with different *para*-substituted phenols (*p*-nitrophenol (**2a**), *p*-chlorophenol (**2b**), phenol (**2c**), *p*-cresol (**2d**), *p*-*t*Bu-phenol (**2e**)). Permittivity dependence of the molecular interactions was obtained in different alcohols as solvents. The results show that **1a** forms stable complexes with **2a**-**e**. The free enthalpy changes show more stable **1a**-**2a** and **1a**-**2b** complexes compared to the stability of **1a**-**2c**, **1a**-**2d** and **1a**-**2e** complexes. Similarly, the entropy changes are significantly different for these two separated groups: the entropy change associated to the formation of **1a**-**2a** and **1a**-**2b** complexes are nearly the same, while large differences in the formation entropy obtained in the other three cases. All the above parameters are slightly changed with the permittivity of the solvents, while the trends of the thermodynamic parameters remained substantially unchanged. To clarify the relevant conformations of the formed complexes DFT/6-31++G calculations were performed where the solvent effect is considered by the PCM (Polarizable Continuum Method). These calculations revealed that the complex formation of **1a** with the **2a** and **2b** phenols is preferably based on Coulomb-interactions between the electron-rich nitro-groups and the electron-deficient carbon rings of calixarenes and those of the **2a** and **2b** guests. Both the experimental and theoretical investigations revealed that no considerable interaction exists between the phenols and **1b.** It is probably due to the ‘locking’ of the calixarene cavity by the bent *O*-ethyl chain. Furthermore, on the bases of these results, any further interaction of phenols with **1a** (or **1b**) can be excluded.



The financial support of the EU and NKTH (Grant GVOP-3.2.1-2004-04-0200/3.0) and the Hungarian Academy of Sciences is highly appreciated.

References

[1] Kunsági-Máté, S., Szabó, K., Desbat, B., Brunneel, J.L., Bitter, I., Kollár,L.: *J. Phys. Chem. B*, **111** (2007) 7218-7223.
[2] Kunsági-Máté, S.; Vasapollo, G; Szabó, K.; Mele, G.; Longo, L.; Kollár L.: *J. Incl. Phen. Macrocycl. Chem.*, **60** (2008) 71-78.

P-39

ENTROPY-DRIVEN COMPLEX FORMATION OF MALVIDIN-3-O-GLUCOSIDE WITH COMMON POLYPHENOLS IN ETHANOL-WATER BINARY SOLUTIONS

Sándor Kunsági-Máté[1], Erika Ortmann[1], László Kollár[2], Martin Pour Nikfardjam[3]

(1) Department of General and Physical Chemistry, (2) Department of Inorganic Chemistry, University of Pécs, Ifjúság 6, H-7624 Pécs, Hungary,
E-mail: kunsagi@gamma.ttk.pte.hu,
(3) State Research Institute for Viticulture and Pomiculture, Traubenplatz 5, D-74189 Weinsberg, Germany

The interaction of malvidin with five polyphenols, also known as “copigmentation”, was investigated by photoluminescence (PL) and quantum chemical (QM) methods in model solutions as a function of the ethanol content. The results show that the entropy change associated with the desolvation of the interacting species prior to complex formation results in several unexpected effects on the copigmentation process. Both the size and the stability of the complexes of malvidin with caffeic and ellagic acid as well as rutin are increased in the presence of higher ethanol concentration. However, although the stability of the malvidin/procyanidin and malvidin/epicatechin complexes also increases with increasing ethanol content, the size of the complexes is drastically reduced when the ethanol content of the solutions exceeds a critical margin of 8%vol. The complex stability shows unexpectedly high dependence on the permittivity of the solvent. Furthermore, the drastic changes obtained in all thermodynamic parameters of the complex formation at around 8%vol. alcohol content can be described by the significantly different composition of the solvation shell of the formed complexes compared to the bulk solutions. QM calculations revealed that at around 8%vol. alcoholic content the mole fraction of ethanol molecules in the solvation shell reaches about 0.5. Independent from further increase in alcoholic content of the solutions this molar fraction remains relatively unchanged. Solvent relaxation measurements support the QM results and highlight that the chroman and syringol parts of malvidin play a different role in the complex formation due to their different electron densities located at the aromatic rings. These results have direct consequences on the winemaking procedure: the winemaker can be assured that the colour in his red wine will be much more stabilized, when the alcohol concentration during fermentation exceeds 8% vol.

This work was funded in part by DAAD (Deutscher Akademischer Austauschdienst) and the MÖB (Magyar Ösztöndíj Bizottság). Financial support of the National Office for Research and Technology (DD-KKV-06-311) is highly appreciated.

References

[1] Kunsági-Máté, S., Ortmann, E., Kollár, L., Nikfardjam, M.P.: *J. Physical Chemistry B* **111** (2007) 11750-11755.
[2] Kunsági-Máté, S., Ortmann, E., Kollár, L., Nikfardjam, M.P.: *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, in press, DOI 10.1016/j.saa.2007.09.021

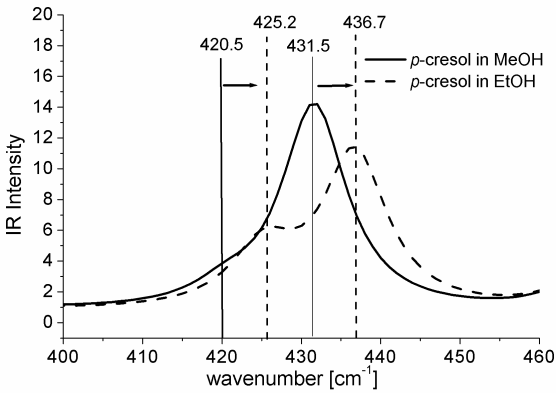
P-40

EFFECT OF MOLECULAR ENVIRONMENT ON THE COUPLING OF MOLECULAR VIBRATIONS OF P-CRESOL

Sándor Kunsági-Máté[1], Sándor Bakonyi[1], László Kollár[2], Bernard Desbat[3]

(1) Department of General and Physical Chemistry, (2) Department of Inorganic Chemistry
University of Pécs, Ifjúság 6, H-7624 Pécs, Hungary,
E-mail: kunsagi@gamma.ttk.pte.hu
(3) Chime et Biochimie des Molécules et Nanosystèmes, Unversity of Bordeaux,
2 rue Robert Escarpit, F-33607, Pessac, France

The competitive thermodynamic and kinetic processes play important role in the formation or dissociation of weak molecular complexes. These complexes are in the focus of host-guest chemistry due to the practical importance in the pharmaceutical, environmental and separation chemistry. While the thermodynamic parameters determine the stability of the complex formed, the kinetic processes determine the rate of the formation-dissociation reaction. Accordingly, in our previous works efforts have been made to examine how the thermodynamic stability was affected by the molecular environment [1]. In the present work theoretical simulations were performed to clarify how the kinetic processes can be affected by the solvent in solution phase. Accordingly, the vibrational spectra of *p*-cresol molecule was calculated at DFT/6-31++G(p,d) level of quantum-chemical calculations and the solvent effect was considered with the Polarizable Continuum Method (PCM). The dynamics of the molecular moving was also examined by molecular dynamics (MD) calculations using AM1 semiempirical Hamiltonian. The results show that the in-plane and out-of-plane molecular vibrations are coupled in the solution phase when the solutions have lower permittivity. We have discussed two representative peaks of the IR spectra of *p*-cresol related to the out-of-plane (420.5 cm^{-1}) and in-plane (431.5 cm^{-1}) vibrations in methanol. These two peaks were slightly shifted to the higher frequencies (to 425.2 cm^{-1} and 436.7 cm^{-1}, respectively) in ethanol. As a parallel effect, these two vibrational modes are coupled in ethanol, and the intensity of the lower frequency peak is increased from 1.12 a.u. to 3.91 a.u. while the higher frequency peak slightly decreased from 13.5 a.u. to 10.3 a.u. These findings could serve as a first step to understand the vibrational coupling of the normalmodes of *p*-cresol.



This work was supported by the Centre National de la Recherche Scientifique (CNRS) and by the Hungarian Academy of Sciences (HAS).

Reference

[1] Kunsági-Máté, S., Szabó, K., Desbat, B., Brunneel, J.L., Bitter, I., Kollár,L.: *J. Phys. Chem. B*, **111** (2007) 7218-7223.

P-41

SENSITIVITY LIMITS OF MATRIX ISOPOTENTIAL SYNCHRONOUS FLUORIMETRY

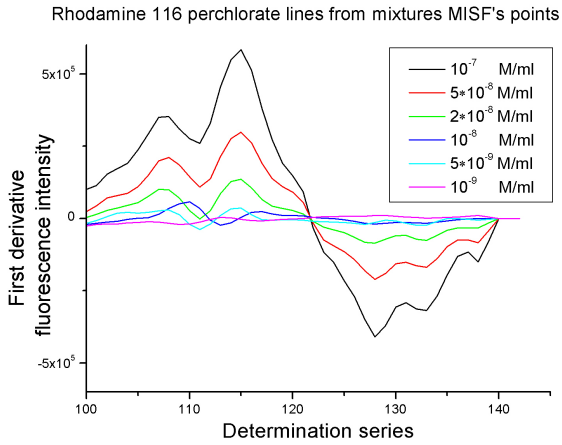
Géza Makkai, Andrea Buzády, János Erostyák

Institute of Physics, Faculty of Sciences, University of Pécs, Ifjúság u. 6. H-7624 Pécs, Hungary, mgeza@ddkkk.pte.hu

Fluorescence emission of different compounds can be well characterized by a three dimensional excitation-emission matrix (EEM) which is generally constructed from single emission or excitation spectra. Using camera recording, the whole EEM can be obtained from one shot. Mixtures have complex EEM reflecting both the fluorescence of the individual compounds and the interactions between them (e.g. energy transfer, quenching etc.). In case of low concentrations, these interactions may be negligible.

Determination of concentrations of closely overlapping compounds gives special difficulties. A powerful and robust method for analyzing these kind of EEMs is the matrix isopotential synchronous fluorimetry (MISF). This may be especially useful for removing fluorescence background and allows the determination of individual compounds in a complex sample. The isointensity points of the mixture give contour lines called isopotential trajectories. Following a trajectory through the wanted compound's fluorescence maximum gives the MISF spectrum. The first derivative of a MISF spectrum of the background is practically zero. Adding the extra compound results in changes of intensity data along the isopotential trajectory, thus the first derivative of the MISF spectrum will reflect the fluorescence of the extra compound. Using the peak-to-peak evaluation, the intensities in a first derivative of MISF spectrum is proportional to the extra compound to be quantitatively determined.

In this work a systematic test of MISF method was done. A mathematical routine for fitting the three dimensional data of EEMs was developed. First, different iteration algorithms were compared. It is a critical point of the method to find a suitable route in the EEM, because the EEMs themselves consist of only the spectrally equidistanced data points. After these, series of both model matrices and real mixtures of three fluorophores were analysed to find the sensitivity limits of MISF. The compounds were rhodamine B, rhodamine 116 perchlorate and rhodamine 6G. Discrimination levels were determined in cases of two- and three-compound mixtures and noisy spectral data. The Figure shows a series of first derivative of MISF spectra.



References

[1] Li, Y.Q., Sui, W., Wu, C., Yu, L.J., *Anal. Sci.* **17** (2001) 167–170.
[2] Murillo, J. A., Alanón, A., *Talanta* **56** (2002) 557–564.
[3] Murillo, J. A., Alanón, A., Fernández, P., *Anal. Chim. Acta* **583** (2007) 55–62.

P-42

LIQUID CHROMATOGRAPHY AT CRITICAL CONDITIONS (LCCC): PRACTICAL APPLICATIONS

Muhammad Imran Malik, Nguyen Viet Cuong, Bernd Trathnigg

Institute of Chemistry, Karl-Franzens-University, Heinrichstrasse 28, A-8010 Graz,Austria.
imranmal@gmail.com

Liquid chromatography is often described by interaction parameter c which may have negative and positive values. Negative value corresponds to size exclusion mode while positive value means adsorption mode. At transition of exclusion and adsorption mode interaction parameter has value equal to zero. At this point polymer chain becomes chromatographically invisible.
In the case of block copolymers this situation can be used very effectively for detection of unwanted homopolymers formed. In this study we demonstrate different applications of critical conditions of certain polymer chain when the other block elutes in exclusion or adsorption regime. If block other than critical is in exclusion regime, block copolymer shall elute before the dead volume of the column. On the other hand, if the second block is in adsorption regime than it shall elute later than critical volume.

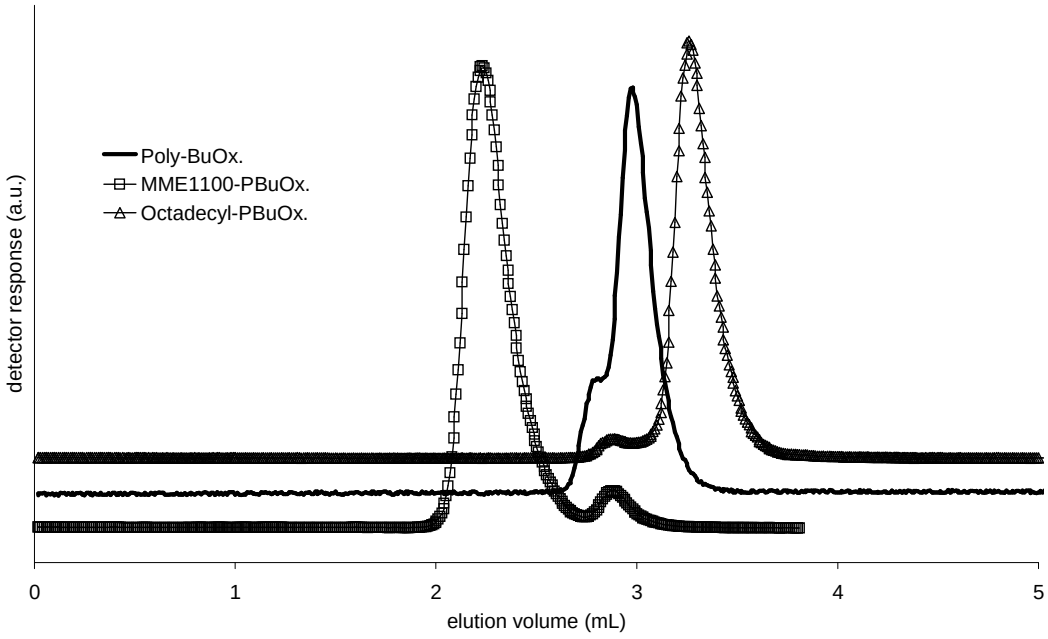


Figure:Application of critical conditions for poly-(butene oxide)

References

[1] B. Trathnigg, Progress in Polymer Science 20 (1995) 615-650
[2] M. I. Malik, B. Trathnigg, C. O. Kappe, Macromolecular Chemistry and Physics 208 (2007) 2510-2524.
[3] M. I. Malik, B. Trathnigg, C. O. Kappe, European Polymer Journal 44 (2008) 144–154.

P-43

DETECTION OF PROGESTERONE FORM CULTURE MEDIA OF *CHORIOCARCINOMA* CELLS USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Erika Marek[1], Piroska Niesz[1], Anita Bufa[1], Viktoria Poór[1], Francoise Raffalli-Matthieu[2], Ferenc Kilár[1]

(1) Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12., 7624, Pécs, Hungary, ferenc.kilar@aok.pte.hu
(2) *Department of Biochemistry and Organic Chemistry, Uppsala Universitet, BMC Husargatan 3., Sweden*

Glutation transferases have important biological function in steroidogenesis in humans and other mammalian species. These enzymes have catalitic efficiency on double-bond isomerizations, for ex. GST A3-3 is currently the most efficient known Δ^{5-} Δ^{4} steroid isomeraze [1.]

The JEG-3 cells (from human choriocarcinoma cells) have been widely used to study steroidogenesis in humans as they express the full complement of well estabilished steroidogenicc enzymes of which many are inducible by classical steroidogenic stimuli. They secrete the synthetized steroids in the culture medium in such quantities that can be measured by gas chromatography.

In this study we developed an optimal method for determining progesteron from culture media. After solid phase extraction, enzym hydrolysis we derivatized the sample with methyloxim in order to stabilize the steroids before high temperature GC analysis.

The work was supported by the grants GVOP-3.2.1-0189, RET 008/2005 and OTKA-NKTH-NI- 68863.

References

[1.] Raffalli-Mathieu F., and Mannervik B. (2005). Human Glutathione Transferase A3-3 Active as Steroid Double-Bond Isomerase. Methods in Enzymology **401**. 265-278

P-44

SOL-GEL COATED ANODIZED ALUMINA FOR SENSOR APPLICATIONS

Ákos Markovics[1,2], Géza Nagy[1], Barna Kovács[1,2]

(1) Department of General and Physical Chemistry, University of Pécs, , Ifjúság str. 6, Pécs
(2) South-Transdanubian Cooperative Research Centre, Ifjúság str.6, Pécs 7624
kovacs1@gamma.ttk.pte.hu

Optical sensors usually consist of a substrate and a chemically sensitive layer. Although in many applications glass is the preferred substrate – e.g. microscope slide or optical fibre - anodized alumina is also suitable. Its surface has excellent reflexivity and the nanometre size pores in the electrochemically prepared oxide-layer assists the adsorption based immobilization of the chemicals. Using different anodizing potential, temperature or electrolyte, a wide variety of pore diameters, layer thicknesses, surface structures can be produced, which affects the sensors dynamic parameters [1].
In most cases, the sensor components are immobilized in a plasticized polymer layer that limits the lifetime of the sensor. These layers suffer from ageing, may peel of after a certain time in operation. Another method for immobilization of the chemicals is the so called sol-gel process [2, 3]. This technology enables the production of glasses at room temperature; a great variety, containing organic or inorganic additives can be made. Depending on the precursors and the method, hydrophobic or hydrophilic, crack-free, stable layers can be fabricated, with longer lifetimes.
In our previous experiments the excellent porosity of anodized alumina was used as substrate for ammonia sensors. The uncoated sensor was unable to use in liquids because the pH changes of the solutions affected the signal. In this work, we report on a sensor, where the anodized alumina substrate is covered by a sol-gel layer preventing the leaching of the indicator and reducing the pH effects caused by the analyte.
Anodized aluminium substrates were prepared by DC method and then immersed in a sol containing the ammonia-sensitive bromocresolgreen (BCG) indicator, methyltriethoxysilane and an acidic catalyst. The gelation process took place in the nanopores of the oxide layer, forming thin and mechanically stable, shiny layers. The sensors were tested in reflection mode by using a fibre-optic photometer. Calibration curves and response curves were taken. The calibration gas mixtures were diluted by air from a 93 ppm calibrating ammonia-nitrogen mixture. The results were compared with our previous experiments, where BCG based ammonia sensors were fabricated with adsorption based immobilization. Sensors made with the sol-gel method has a dynamic range of 1-100 ppm ammonia and a response time less than 30 s, while the regeneration takes half an hour.

References

[1] Th.Dimogerontakis, I.Tsangaraki-Kaplanoglou, The role of aluminum anodizing conditions on the effect of the additive light green Thin Solid Films 402 (2002) 121–125
[2] A. Persad, K.-F. Chow, W. Wang, E. Wang , et al. Bocarsly, Investigation of dye-doped sol–gels for ammonia gas sensing, Sensors and Actuators B 129 (2008) 359–363
[3] A Lobnik, O. S. Wolfbeis, Sol-gel based optical sensor for dissolved ammonia, Sensors and Actuators B 51 (1998) 203–207.
[4] A. Markovics, G. Nagy, B. Kovacs, Reflection based sensor for gaseous ammonia, Sensors and Actuators B, (2008) accepted.

P-45

ION-EXCHANGE MONOLITHIC STATIONARY PHASES PREPARED BY RING-OPENING METATHESIS POLYMERIZATION

Agnes Mautner[1], Christina Gatschelhofer[1], Franz Reiter[1], Christoph Magnes[1], Michael R. Buchmeiser[2], Thomas R. Pieber[1,3], Frank M. Sinner[1]

(1) Institute of Medical Technologies and Health Management, JOANNEUM RESEARCH, Auenbruggerplatz 20, A-8036 Graz, Austria
(2) Leibniz Institute for Surface Modification e.V., Permoserstr. 15, D-04318 Leipzig, Germany
(3) Department of Internal Medicine, Diabetes and Metabolism, Medical University Graz, Auenbruggerplatz 15, A-8036 Graz, Austria
frank.sinner@joanneum.at

To cope with the increasing separation requirements of the fields of proteomics and drug discovery, separation media with smaller inner diameters, higher separation performance and better sensitivity are needed. With the need for capillary columns possessing high separation performance the concept of monoliths is steadily becoming more appealing [1]. In recent years there has been considerable interest in developing monolithic columns bearing functional groups. Ring-Opening Metathesis Polymerization (ROMP) offers the unique possibility to prepare functionalized monolithic columns utilizing the "living" character of the initiator [2,3].
The preparation of functionalized monoliths via ROMP is conducted in a two step process. In the first step the monolithic structure is formed by polymerization of norborn-2-ene (NBE) and 1,4,4a,5,8,8a-hexahydro-1,4,5,8,-exo,endo-dimethanonaphthalene (DMN-H6) using a Grubbs-type initiator within the confines of a fused-silica capillary of 200 µm inner diameter. In the second step, the still active initiator sites, located at the surface of the structure-forming microglobules, are used for grafting functional groups onto the monolithic backbone by flushing the monolith with any NBE based functional monomer.
We report on the preparation of ROMP-derived monolithic capillary column for anion-exchange chromatography using two different grafting techniques. A detailed evaluation of the effects of the preparation conditions of two different grafting techniques on the ion-exchange capacity will be shown. The influence of the derived capacity on the chromatographic behavior will be demonstrated by the separation of oligonucleotides.

References

[1] Wu, R.; Hu, L.; Wang, F.; Ye, M.; Zou, H. *J. Chromatogr.A* **2008**, *1184*, 369-92.
[2] Sinner, F.M.; Buchmeiser, M.R. Angew.Chem. 2000, 112, 1491-94
[3] F. M. Sinner and M. R. Buchmeiser, Macromolecules 33 (2000) 5777.

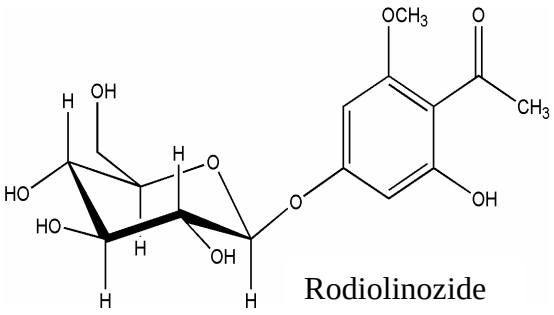
P-46

PHYTOCHEMICAL INVESTIGATION OF *CALLUNA VULGARIS* (L.) HULL USING LC/PDA/ESI-MS ANALYSIS

Marlene Monschein[1], Jacobo Neira Inglesias[2], Olaf Kunert[1], Franz Bucar[1]

(1) *Institute of Pharmaceutical Sciences, University of Graz, Universitätsplatz 4/1, A-8010 Graz, Austria*
(2) Instituto de Investigaciones Marinas (CSIC), Av. Eduardo Cabello 6, 36208 Vigo, Spain.
ma.monschein@edu.uni-graz.at

Calluna vulgaris (L.) HULL (Common heather, Ericaceae) is a small evergreen branched shrub growing almost in entire Europe and in West-Siberia. Traditional folk and experiential medicines apply herbs of *C. vulgaris* in therapy of various diseases, such as renal and urinary tract disturbances, gastrointestinal, respiratory and sleep disorders [1,2]. Aerial, herbaceous plant material was subjected to a detailed phytochemical analysis by chromatographic means (TLC, NPLC (Sephadex LH-20), LC/PDA/ESI-MS). Fractions were analyzed by HPLC using a reverse-phase column (Phenomenex Synergi Hydro-RP) as a stationary phase and a gradient of water containing 0.5% acetic acid and acetonitrile : water (50:50) containing 0.5% acetic acid as mobile phase. Under the applied MS conditions (ESI, negative ion mode), tiliroside (kaempferol-3-O-(6´´-p-coumaroyl)-β-D-glucoside), p-cumaroylquinic acid, an apigenin hexuronolactone, a quercetindimethylether hexoside, mono-, di- and tetraacetylarabino-glucosides of quercetin and kaempferol as well as rodiolinozide could be identified in this genus for the first time. Rodiolinozide, 1-[4-(β-D-glucopyranosyloxy)-2-hydroxy-6-methoxyphenyl]-ethanone, was isolated from the ethyl acetate extract and structurally elucidated using UV, 1- and 2-dimensional NMR and LC-MS analyses.
This acetophenone was previously only reported from *Artemisia* and *Rhodiola* sps. [3,4].



Rodiolinozide

Acknowledgements
The Dr.-Heinrich-Jörg-Stiftung and the Forschungsmanagement-und service is gratefully acknowledged for financial support.

References
[1] Länger R., Kubelka W. (2001) Phytokodex. Krause & Pachernegg GmbH. Gablitz.
[2] Königshofer I. (2004) Erhebung von Heilkäutern im Naturpark Sölktäler, Diploma thesis, University of Graz.
[3] Singh A.K., Pathak V., Agrawal P.K. Phytochemistry 1997, 44, 555-557.
[4] Satsyperova I.F., Kurkin V.A., Zapesochnaya G.G., Pautova I.A., Avdeyeva E.V. Rastitel´nye Resursy (1995), 31, 27-21.

P-47

DETERMINATION OF THE ANTIMALARIAL DRUG ARTEMISININ BY USING MODIFIED CARBON ELECTRODES

Susanne Hauser, Sonja Mödritscher, Chhanda Debnath, Astrid Ortner

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, University Graz, Schubertstrasse 1, A-8010 Graz

Artemisinin is a sesquiterpene endoperoxide, which is isolated from the herb of the Chinese medicinal plant *Artemisia annua* [1]. It is a potent antimalarial drug against the resistant strains of *Plasmodium falciparum* [2]. The antimalarial action of artemisinin appears to be mediated by the generation of free radicals from the endoperoxide bridge of the drug [3]. *Artemisia annua* seems to be the unique natural source of artemisinin. Production of artemisinin in this plant is usually in the range of 0.01 - 0.4%, but some clones produce more than 1% dry weight [4].

Analysis of artemisinin is quite challenging as it is a thermolabile compound, shows no chromophoric or fluorophoric groups, the concentration in the plant is low and other compounds in the crude plant extracts can interfere in its detection. Several analytical methods have been developed (GC, TLC, HPLC, CE, ELISA, SFC) for the determination of artemisinin in *Artemisia annua* [4]. All these systems have been well adapted, however they have some limitations.

Due to the fact that artemisinin contains an endoperoxide (-O-O-) group, which is electrochemically active, it can be determined by using electroanalytical methods at various electrodes [5, 6]. Modifying electrodes provides the opportunitiy of lowering the overpotential and improving the transfer characteristics. Because of this advantage the aim of our work was to develop a bulk modified carbon electrode for the determination of artemisinin. As modifier for the carbon electrode hemin (iron protoporphyrin IX) was selected.

In order to develop the hemin modified carbon electrode, which ought to be simple in its preparation, accurate and applicable in complex plant materials, the construction and preparation of the electrode as well as the conditions for the measurements were optimized. In phosphate buffer solution at pH 7 the developed hemin modified carbon electrode showed a significant catalytic activity in the presence of artemisinin at about -380 mV vs. Ag/AgCl by using cyclic and differential pulse voltammetry. Strict linearity between artemisinin concentration and peak height was observed in 4.8×10^{-6} - 7.8×10^{-5} mol/L concentration range ($R = 0.9991$) when applying differential pulse voltammetry. The detection limit of artemisinin was calculated to be 1.4×10^{-6} mol/L. The hemin modified electrode can be used to determine artemisinin in complex plant materials.

References:

[1] Qinghaosu Aantimalaria Coordinating Research Group, Chin. Med. J., 92, 1979, 811.
[2] Wernsdorfer H.W.; Expert Review of Anti-infective Therapy 2, 2004, 181-196.
[3] Robert A., Meunier B.; J. Am. Chem. Soc. 1997; 119: 5968-5969.
[4] Christen P., Veuthey J.-L; Curr. Med. Chem. 2001; 8:1827-1839.
[5] Katritzky Zhou Z.J., Yang P.H., Feng D.X., Zhu Y.T., Zhang M.Y.; Yunnan Daxue Xuebao 2003; 25:144-147.
[6] Debnath C., Haslinger E., Ortner A.; NPC 2006; 6: 487-494.

P-48

MICROSIZED AMPEROMETRIC BIOSENSORS FOR CLINICAL DIAGNOSIS

Lívia Nagy, **Géza Nagy**

Research Group for Chemical Sensors, Faculty of Sciences, University of Pecs, Ifjúság u. 6, 7426 Pécs, Hungary (lnagy@ttk.pte.hu)

Amperometric sensors, specially microsized amperometric biosensors are successfully employed in different areas of experimental life sciences, as well as in clinical diagnosis. In our experiments amperometric sensors for determination of clinically important species like glucose, putrescine and reactive oxidative species (like superoxides, H_2O_2 or nitric oxide) are prepared and tested. Further more efforts are made for developing new measuring methods for extending their measuring range, improving selectivity and sensitivity.Recently we worked out a new detection method, the periodically interrupted amperometry (PIA), for lowering the detection limit of membrane coated electrodes, providing more sensitive detection.

As it is well known, the amperometric enzyme electrodes are made of an amperometric sensing element and different layers coated its surface. While in use the electrode potential kept constant and the diffusion current through that stationary layers are detected. The concentration of electroactive species formed in the enzyme catalysed reaction is decreased by the continuous action of the electrode process. In this way the stationer layer at the surface of the sensing element gets more or less depleted (exhausted).

It was expected, that periodic interruption of the electrolysis, that would allow reloading the layer at the vicinity of the sensing element could improve the performance of enzyme sensors.

Along this line, we developed a novel PIA measuring method, introduced here showing the enhanced performance of putrescine electrode. Elevated local concentration of putrescine is assumed as indication of bacterial infection, while in blood rising trace level of putrescine is among cancer markers. The electrode function is based on the following reaction. The reaction product H_2O_2 is electrochemically active component.

$$\text{Putrescine} + H_2O + O_2 \xrightarrow{\text{Putrescine oxidase}} 4\text{-Aminobutyraldehyde} + NH_3 + H_2O_2$$

The PIA measurements with three electrode systems were made using Autolab PGSTAT12 workstation (Eco-Chemie BV, Utrecht, Holland). Platinum disc electrodes were used for preparation of the enzymatic working electrode (0,78 mm^2, and implantable 1,75 mm^2 microchip). Electrochemically prepared poly-(m-phenylenediamine) layer on the platinum disk provided selective H_2O_2 detection. Immobilized putrescine oxidase enzyme containing film was attached on the electrode as reaction layer, selective catalyst. Pt needle served as counter electrode and commercially available Ag/AgCl for reference one. In vitro experiments in PBS buffered solutions as well as in, in complex matrices like blood and plasma were performed.

Conventional amperometric detection and PIA detection were compared. The signals obtained with the optimized PIA program was 1000 times higher than the current obtained with the classical amperometry. Results show that the PIA detection extends the measurement range of membrane coated amperometric sensors. In clinical samples putrescine is in lower μM range. Therefore lowering the detection limit is an important step forward.

The authors would like to thank Hungarian Scientific Research Found for financially supporting this research with grant T 049502.

References

[1] Nagy, G. and Nagy L.: *Anal. Lett*, **40** (2007) 3-38.

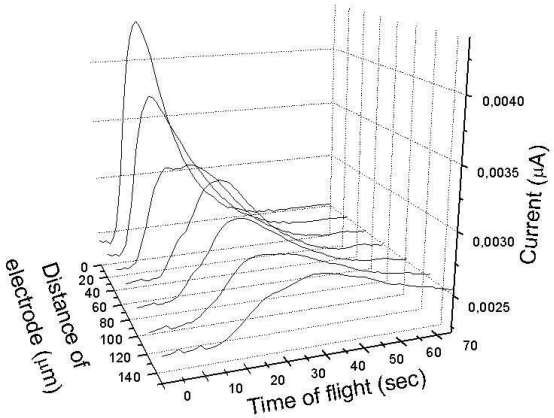
P-49

RECENT RESULTS WITH SECM DIFFUSION COEFFICIENT MEASURING

Lívia Nagy, Gergely Gyetvai, Géza Nagy

General and Physical Chemistry Department, Faculty of Sciences, University of Pécs, Ifjúság útja 6., 7632 Pécs, Hungary

The diffusion coefficient of different molecules in different media is an important parameter in several areas. High number of electrochemical methods are available for measuring it.
A simple method, based on special capability of Scanning Electrochemical Microscopy (SECM) for the accurate measurement of diffusion coefficient, have been worked out in our laboratory.
It is based on detecting the concentration – time transients with appropriate electrochemical micro-sensor positioned at the close vicinity of a miniature dose source device. At given time (t_i) a small dose of the investigated species is introduced. The $\Delta t_{max} = (t_{cmax}-t_{í}$) value and the distance ($d = x+ \Delta x_n$) between the source and the detector microelectrode are used for calculation of D. While the original set distance (x) can not be accurately measured in the micrometer scale, the tip travel distance (Δx_n) of the microscope is well defined. Collecting a few $\Delta t_{max} – (x+ \Delta x_n)$ data pairs reliable value of the diffusion coefficient can be obtained. The procedure is simple and does not require knowing the dose size introduced.
In the work to be presented the basic principle of the new method will be presented, and the values obtained with it will be compared with diffusion coefficient values obtained using conventional electrochemical methods. In the experiments micro size SECM tips, enzyme sensor, ionselective electrodes and conventional size voltammetric electrodes were used. Diffusion coefficients of practically important molecules in solutions and gels will be presented.



References

Csóka B., Nagy G.: *Journal of Biochemical and Biophysical Methods*, **61** (2004) 57-67

P-50

POSSIBILITIES OF DETERMINATION OF DIFFERENT DYE SUBSTANCES USING CIEF-MS ANALYSIS

Csilla Páger, Ferenc Kilár

Institute of Bioanalysis, Faculty of Medicine, University of Pécs,
P.O.Box 99, H-7643 Pécs, Hungary

Capillary isoelectric focusing (cIEF) is a rapid, high performance analytical method that separates amphoteric compounds based on their isoelectric point (pI). We have previously worked out a cIEF technique based on an injection protocol, which allows the separation of amphoteric compounds outside the pH region of the ampholytes applied in the capillary. Our intention was to connect this "sandwich" cIEF method with mass spectrometric (MS) detection.

Substituted aminomethylphenol dyes (isoelectric points: 5.3, 6.4, 6.6, 7.9 and 10.4) and anthranilic acid (2-aminobenzoic acid) derivatives (isoelectric points: 2.7, 3.0, 3.5, and 4.0) have been separated employing ampholytes with narrow and broad pH ranges in uncoated capillaries. With the use of the anthranilic acid derivatives we would like to visualize the pH gradient at pH 4 and below. In this case the substances may abandon the zone of the ampholytes, what may be advantageous in detection.

In order to combine the cIEF method with mass spectrometric detection volatile solution were applied as anolyte and catholyte (formic acid and ammonium hydroxide), since volatile solutions are preferred in MS analysis. This modified cIEF method in combination with the MS detection was successfully achieved showing the separation of the pI markers.

In the future we are going to employ this cIEF-MS method for the separation of proteins, but first of all we have to find a pH stable capillary coating where the adsorption of the protein onto the capillary wall is greatly reduced.

The work was supported by the grants GVOP-3.2.1-0168, RET 008/2005 and OTKA-NKTH-NI- 68863.

P-51

THE EFFECT OF VIBRATION DYNAMICS OF SWCNTS ON THEIR ION AND MOLECULE TRANSPORT

Beáta Peles-Lemli[1], László Kollár[2], Géza Nagy[1], Sándor Kunsági-Máté[1]

(1) Department of General and Physical Chemistry,University of Pécs, Ifjúság 6, H-7624 Pécs, Hungary, blemli@gamma.ttk.pte.hu
(2) Department of Inorganic Chemistry, University of Pécs, Ifjúság 6, Pécs, H-7624, Hungary

Single-walled carbon nanotubes (SWCNTs) have a fascinating ability to encapsulate atoms, ions and small molecules. Although their special properties, *e.g.* their one-dimensional form, would make them highly attractive materials, the very complex behaviour of the ion and molecule transport still hinders their wide application. In our previous work [1] the Molecular Dynamics (MDs) simulations were found fruitful testing techniques to analyze the dynamic processes of aromatic materials at molecular level. Accordingly, in our preliminary investigations the two representative types of carbon nanotubes were studied by temperature dependent MDs calculations. Our results highlighted that in contrast with the results of Sholl et al. [2] derived from static representation, the transport within the nanotube is preferably determined by the acoustic waves passing through the nanotube. We have found, that the longitudinal wave of the conformation change travels along the longitudinal axis of the nanotube at the rate of 20 nm/ps. Considering this result with the diameter of the investigated SWCNTs, the speed of the investigated wave generated by the diameter changes of SWCNT is about 4.6×10^{-1} cm^2s^{-1}, which value is in the range of the calculated diffusion coeffitients of Sholl et al. [2]. This result supports that the pulsation of the nanotube can control the transfer of any kind of particles whose shape fits to an actual conformation of the nanotube wall. Our recent work shows the possibility to form a stationary wave by interference of longitudinal waves mentioned above. This effect suggests the presence of special situations, where the ion and molecule transport in these nanoscale channels is completely blocked by the stationary wave on the longitudinal direction of the nanotube. In this case the particles are located *e.g.* in the circular section of the tube. Furthermore, our results indicate correlation between the diameter and the length of the SWCNTs during the formation processes of the stationary waves. In addition to the work of Sholl et al. [2], our results highlighted that the energy transfer between the nanotube and the diffusing molecules depends also on the geometry changes of the SWCNT. The results described above might serve to develop the theories related to the tubular ion and molecule transports.

The financial support of the EU (Grant GVOP-3.2.1-2004-04-0200/3.0) and of the National Office for Research and Technology (Grant OMFB-01486/2006) are highly appreciated.

References

[1] Lemli,B., Peles, J., Kollár, L., Nagy, G., Kunsági-Máté, S., *Supramol. Chem.* **18(3)** (2006) 251-256.
[2] Chen, H., Johnson, J.K., Sholl, D.S., *J. Phys. Chem. B* **110** (2006) 1971-1975.

P-52

SOLUBILIZATION OF SWCNTS: PERMITTIVITY-DEPENDENT CARRIER PROPERTY OF ANILINE DERIVATIVES

Beáta Peles-Lemli[1], Péter Ács[2], László Kollár[2], Sándor Kunsági-Máté[1]

(1) Department of General and Physical Chemistry,University of Pécs, Ifjúság 6, H-7624 Pécs, Hungary, blemli@gamma.ttk.pte.hu
(2) Department of Inorganic Chemistry, University of Pécs, Ifjúság 6, Pécs, H-7624, Hungary

Although the amazing physical and chemical properties of single-walled carbon nanotubes (SWCNTs) preserve their success in wide-scale chemical applications, their wide-spread use is inhibited by their very low solubility in aqueous or most organic media. Nevertheless, a recent work showed [1] that aniline itself can solve the nanotubes by forming a charge-transfer complex. Although SWCNTs solubilized in aniline have increased attention, questions still remained open whether the aniline, used in composition with other solvents, could serve as carrier molecule in the solubilization of nanotubes. According to our previous work [2] it was obvious to assume that the interaction between the nanotube and aniline is highly affected by the permittivity of the molecular environment. In this work the aniline – SWCNT interactions and the carrier property of aniline related to the SWCNTs were investigated by photoluminescence (PL) methods and Raman measurements in different solvents. In order to clarify the solvation mechanism of SWCNTs in aniline, different aniline derivatives (aniline, 2,6-dimethyl-aniline and *N*,*N*-dimethyl-aniline) were chosen in the present work. For preliminary investigations the solvent effect on the interaction of the above aniline derivatives with SWCNT were studied using five different solvents (carbon tetrachloride, *n*-butanol, *n*-propanol, ethanol and methanol). These compounds are representative for wide-scale, medium and low-permittivity solvents. Two particular aspects were considered, namely, *i)* how the aniline interacts with carbon nanotubes, and *ii)* how the permittivity of the molecular environment affects the aniline – nanotube interaction. During these measurements some interesting permittivity-dependent changes in the PL and Raman spectra were found. These changes give relevant signal for the aniline – nanotube interactions, but, in parallel, highlight that the aniline – nanotube interactions are also affected by the conformational change of the aniline molecule. The latest effect seems to be driven by the permittivity of the solvent used. These results might contribute to the development of new non-covalent-interaction-based solubilization procedures of SWCNTs in aqueous or common organic media, which is one of the requirements of the success of carbon nanotubes in wide-scale chemical applications.

The financial support of the European Union – Hungarian National Development Program (Grant GVOP-3.2.1-2004-04-0200/3.0 and GVOP-3.2.1-2004-04-0168/3) is highly appreciated.

References

[1] Sun, Y., Wilson, S.R, Schuster, D.I., *J. Am. Chem. Soc*. **123** (2001) 5348-5349.
[2] Kunsági-Máté, S., Bitter, I., Grün, A., Nagy, G., Kollár, L., *Anal. Chim. Acta* **461** (2002) 273-279.

P-53

SURFACE FRACTAL AND STRUCTURAL PROPERTIES OF LAYERED CLAY MINERALS MONITORED BY SMALL ANGLE X-RAY SCATTERING AND LOW TEMPERATURE NITROGEN ADSORPTION EXPERIMENTS

Tímea Pernyeszi[1], Imre Dékány[2]

(1) Department of Analytical and Environmetal Chemistry, Faculty of Science, University of Pécs, 6 Ifjúság, H-7624 Pécs, Hungary
(2) Department of Colloid Chemistry and Nanostructured Materials Research Group of the Hungarian Academy of Sciences, University of Szeged, H-6720 Szeged, 1 Aradi Vt., Hungary

Small-angle X-ray scattering of the clay minerals kaolinite, montmorillonite and illite was studied with a compact Kratky camera. From the scattering function, the correlation length, the Porod constant and the specific surface area were determined. The scattering functions also led to the surface fractal dimension. The pore volume distribution, the adsorption/desorption hysteresis, the specific surface area and the surface fractal dimension of the same samples were also determined by nitrogen adsorption at 77 K.

Keywords: clay minerals, kaolinite, illite, montmorillonite, small-angle X-ray scattering, nitrogen adsorption

P-54

ANALYSIS OF VOLATILE FATTY ACIDS TO OPTIMIZE THE PROCESS OF FERMENTATIVE BIOGAS PRODUCTION

Friedrich W. Pichler[1,2], A. Ranz[1], Ernst P. Lankmayr[1]

(1) Institute of Analytical Chemistry and Radiochemistry, University of Technology Graz, Technikerstr.4, 8010 Graz, Austria
(2) Chair of General and Analytical Chemistry, University of Leoben, Franz-Josef-Str. 18, 8700 Leoben, Austria

The shortening of mineral oil makes the utilization of alternative sources of energy a crucial necessity. One strategy to become independent of fossil fuels is the production of biogas, by the anaerobic fermentation of organic matter [1]. The produced gas consists of high amounts of CH_4 - beside CO_2 and traces of H_2S, H_2 and NH_3 – which can serve as fuel or may be converted to electric or thermal energy. Renewable resources like maize silage, swine manure and various sorts of organic waste can be used as a "substrate" for the production of biogas. These materials constitute energy sources that will never be used up. Furthermore, the utilization of biogas, contrary to petrol, is a CO_2 neutral, sustainable process, thus all the CO_2 emitted upon CH_4 combustion is "recycled" during the photosynthetic buildup (= growth) of new organic matter. Therefore production of energy from biogas does not add to the global greenhouse effect. Numerous biogas plants are already operating all over the world. Their performance, naturally, depends on the fermentation process, and, due to varying substrate composition, fluctuates strongly over time [2]. However, there is a demand to improve the control mechanisms of the fermentation process.
Short-chain fatty-acids [1] are metabolites, that form during the fermentation process, and are known to strongly inhibit the biogas production leading to markedly decreased methane yields and may even cause the breakdown of the whole process, if no preventive measures are taken. Therefore, a robust method to monitor the evolvement of these intermediates is considered a key tool for efficient process regulation.
In this work, a robust analytic method to quantify these fatty-acids has been developed.
The technique used to identify type and amount of the relevant volatile fatty-acids in the fermentation broth, is the static headspace extraction. Matrix effects on the dispersion of the analyte between vapor phase and liquid /solid phase, such as adsorption or absorption, were ruled out via "multiple headspace extraction" experiments [3]. After esterification of the acids, the vapor phase was analyzed without further sample preparation via HS-GC-MS. The new method was optimized and validated via "Validata" [4].
This method can be used as an efficient tool for monitoring, regulating and optimizing the process of biogas production. It markedly simplifies sample preparation and reduces analysis time compared to analytical methods used to date.

References

[1] Braun, R. 1982. Biogas – Methangärung organischer Abfallstoffe Grundlagen und Anwendungsbeispiele. Springer Verlag
[2] Fachagentur Nachwachsende Rohstoffe e.V. 2005. Handreichung Biogasgewinnung und -nutzung.
[3] Strassnig S., Lankmayr E.P.; Elimination of matrix effects for static headspace analysis of ethanol; Journal of Chromatography A; **849** (1999) 629-636.
[4] Validata Version3.02.54ger (Wegscheider-Rohrer-Neuböck. Leoben, Österreich)

P-55

SENSITIVE INSULIN ANALYSIS IN INTERSTITIAL FLUID: INFLUENCE OF MOBILE PHASE ADDITIVES ON INSULIN B CHAIN LOADING EFFICIENCY IN CAP-HPLC/MS^2

Karin Pickl[1], Olivia Schnitzer[1], Christoph Magnes[1], Thomas R. Pieber[1,2], Frank M. Sinner[1]

(1) Institute of Medical Technologies and Health Management, Joanneum Research, Auenbruggerplatz 20, 8036 Graz, Austria
(2) Department of Internal Medicine, Medical University Graz, Auenbruggerplatz 15, 8036 Graz, Austria
frank.sinner@joanneum.at

In recent years, new insulin analogues with altered pharmacokinetic (PK) properties providing improved glucose control in diabetes have been developed [1]. The measurement of insulin in body fluids (blood, interstitial fluid (ISF)) for the purpose of PK profiling is currently done by immunoassays. Such methods however are limited by cross-reactivity with structurally related peptides, a lack of assays specific for individual insulin analogues, and the inability to assay multiple insulins simultaneously . A sensitive cap-HPLC/MS^2 method allowing simultaneous analysis of human insulin and several insulin analogues (insulin lispro, insulin aspart, insulin glulisine, insulin glargine, bovine or porcine insulin) with no cross-reactivity issues has been recently developed for the determination of physiological insulin concentrations in human serum (36 – 1500 pM) [2]. The sensitivity of this method must however be further optimized for the analysis of ISF, the actual insulin action compartment in the human body [3] since physiological insulin concentrations are in the range of 20 -100 pM [4] in this fluid and the available ISF sample volume for analysis is limited (2 – 100 µl).
Sensitivity can be improved by the use of mobile phase additives which increase the loading efficiency of peptides in HPLC analysis. The current set-up uses 0.05 % trifluoroacetic acid (TFA) and 1% acetic acid (AA) in the loading mobile phase. According to the literature [5,6], higher loading efficiencies can be obtained by using heptafluorobutyric acid (HFBA) instead of TFA. We therefore tested the effect of varying the concentrations of the mobile phase additives, TFA and HFBA, on the loading efficiency of human insulin and insulin analogue B chains. Results will be presented for two different matrices: Matrix A (1% human serum albumin in starting mobile phase) which closely mimics interstitial fluid and Matrix B (10000 fmol/µl insulin detemir in starting mobile phase) which constitutes an optimum matrix with respect to insulin B chain stability in solution.

References

[1] J. Brange et al, Advanced Drug Delivery Reviews **35** (1999) 307-335
[2] K. Pickl et. al, "New strategy for insulin and insulin analogs quantification - MRM detection for insulin B chain", 55nd ASMS Conference, June 3rd – 7th, 2007, Indianapolis (Indiana, USA),
[3] M. Bodenlenz, et al; Am.J.Physiol Endocrinol.Metab **289** (2005) E296-E300.
[4] M. Sjostrand et al, Diabetes **51** (2002) 2742-48.
[5] M. Shibue et al, J.Chromatogr.A **1080** (2005) 58-67.
[6] W. Walcher et al, J.Chromatogr.A **1053** (2004) 107-17.

P-56

ENANTIOSEPARATION USING DYNAMICALLY COATED REVERSED PHASE COLUMNS

Elfriede Pittler, Martin G. Schmid, Gerald Gübitz

Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens-University, Universitätsplatz 1, A-8010 Graz, Austria

About 60% of the drugs in use are chiral and only 48% are administered as pure enantiomers. The separation of chiral drugs has become to a topic of increased pharmaceutical interest since in most cases only one of the enantiomers exhibits the desired pharmaceutical activity. Whereas the pharmacological inactive enantiomer can cause unwanted side effects, antagonistic activities or even toxic effects. Therefore, there is a crowing demand to develop analytical methods for enantioseparation.

HPLC has become one of the most commonly used chromatographic methods in the field of chiral separations. A new trend is the use of monolithic columns, which exhibits advantages over packed LC columns.

Our work deals with the preparation and application of dynamically coated chiral stationary phases for HPLC. Dynamical coating of a hydrophobic chiral selector to a reversed-phase material is inexpensive and easy to prepare. Commercially available monolithic RP-18 HPLC columns were dynamically coated by pumping an aqueous solution containing the chiral selector through the column. In a previous work [1], we presented the possibility of applying the chiral separation principle of ligand-exchange on dynamically coated monolithic columns using N-alkyl-derivatives of L-4-hydroxyproline as chiral selectors. In the present work, we show that also a long-chain N-alkyl-derivative of the macrocyclic antibiotic vancomycin can be used for this purpose. With these two chiral selectors the enantioseparation of a broad spectrum of amino acids, α-hydroxy acids, dipeptides, tripeptides as well as dansyl amino acids was possible.

Reference

[1] M.G. Schmid, K. Schreiner, D. Reisinger, G. Gübitz, J. Sep. Sci, 29 (2006) 1470-1475

P-57

BROMINATED FLAME RETARDANTS - AN EXTRACTION FROM POLYMERS

Andreas Ranz[1], Eveline Maier[1], Christian Trampitsch[2], Ernst P. Lankmayr[1]

(1) Graz University of Technology, Institute of Analytical Chemistry and Radiochemistry, Technikerstrasse 4, 8010 Graz, Austria
(2) Anton Paar GmbH, Anton-Paar-Strasse 20, 8054 Graz, Austria
andreas.ranz@tugraz.at

Decabromodiphenyl ether (DecaBDE) is used as an additive flame retardant mainly in plastics and textile applications. Each year, more than 7.500 tonnes of DecaBDE are imported into the European Union.
But contrary to its lower brominated congeners, this flame retardant is not banned by the decision 2005/717/EG of the European Union. The question of the bioavailability and bioaccumulation of this second most used flame retardant worldwide becomes subject of more intense research. Due to the fact that more and more studies are published indicating time trend increasing of concentrations in different environmental matrices and also in humans, it is astonishing that no standard analytical procedures have been set for these analytes; especially the determination directly from the main originator, from the relevant polymers. Thus, the necessity of a monitoring of DecaBDE needs to be pointed out. Mainly used in electrical and electronic appliances, a determination directly in these materials according also due to the Waste Electrical and Electronic Equipment Directive (WEEE Directive, 2002/96/EC) is necessary.
In the present study, a method for the determination of DecaBDE in polymers has been developed, optimized and validated. The concentration at which DecaBDE has to be measured in polymers is generally above 1 ppm and special emphases have been given to an accurate extraction. The challenge in polymer extraction is not only to reach high recoveries of investigated analytes, but also to create an easy-to-handle method.
Quantification was performed by means of HPLC-DAD. In order to achieve comparable data, extraction was also performed with classic Soxhlet extraction. To compare these results with an independent technique, microwave-induced oxygen combustion and ion chromatography complete this study.

P-58

DETERMINATION OF FATTY ACID DERIVATIVES IN FUEL OIL

Andreas Ranz, Eveline Maier, Ernst P. Lankmayr

Graz University of Technology, Institute of Analytical Chemistry and Radiochemistry
Technikerstrasse 4, 8010 Graz, Austria
andreas.ranz@tugraz.at

Fuel oil plays one of the most important roles as thermal energy source in domestic application. Due to this large use, a careful monitoring of the grade and the deviation of the quality is required. Recently it has been observed that the presence of polar fatty acid derivatives is leading to problems in the fuel injection systems and consequently can cause a damage of the engine.
Here we present a fast and reliable working procedure, which allows the group determination of these polar compounds, namely glycerides, soaps and fatty acids. A sample preparation succeeded, which accomplishes the extraction, the clean up and the derivatization in one single reaction vessel.
Derivatives were methylated with acetyl chloride and methanol and quantified with gas chromatography mass spectrometry. The complexity of heating oil requires a careful elaboration of the clean up and the extraction procedure. Owing to the novelty of this procedure, an accurate investigation and optimization of influential parameters were performed. Optimum conditions for the solid phase extraction with polar sorbents were provided for all target compounds. Additionally, the necessity of the clean up is shown with the relation between investigated analytes and matrix effects. Finally, method performance was verified with an accurate validation and analyses of field samples.

P-59

SIMULTANEOUS DETERMINATION OF S-ADENOSYL-METHIONINE AND S-ADENOSYL-HOMOCYSTEINE IN TISSUE BY CAPILLARY ELECTROPHORESIS

Marzena Kaniewska, Stephanie Schweiger[1], Marianne Huber[1], Martin Pichler[2], Peter M. Abuja[2], and Martin Schmid[1]

[1] Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens University of Graz, Austria
[2]Institute of Pathology, Medical University of Graz, Austria
martin.schmid@uni-graz.at

S-adenosyl homocysteine (SAH) and S-adenosyl methionine (SAM) play an important role in the cellular methionine and glutathione metabolism, and in cellular methylation processes. In alcoholic and non-alcoholic liver disease (steatohepatitis) decreased SAM and increases SAH values are frequently observed, and consequentially supplementation with SAM was suggested for therapy. In a mouse-model (male Swiss Albino, 6-8 weeks old) where major features of human steatohepatitis were induced by feeding a diet containing 1.5% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) we observed downregulation of both methionine adenosyltransferase and adenosylhomocysteinase by quantitative RT-PCR, which prompted us to investigate the corresponding changes in SAM and SAH concentrations.
A simple and rapid method was developed to simultaneously quantify SAM and SAH in soft tissue by capillary electrophoresis. The procedure involves tissue homogenization, protein-precipitation in sulfosalicylic acid and single-step exctraction of the watersoluble constituents of the tissue. In case of tissue rich in lipids, extracts are washed with chloroform. Capillary electrophoresis is performed without further sample workup leading to simultaneous detection of SAM and SAH using a background electrolyte consisting of phosphate or TRIS. UV absorbance was monitored at 200 nm or 257 nm. Response factors, repeatability precision, day-to-day variation, levels of detection and quantitation, and recovery were determined. Furthermore, sample storage conditions were examined with respect to stability of SAM and SAH at -70°C, and stability dependence on storage pH.
We found that, compared to controls, in animals fed DDC for three and 7 days, SAM concentration decreased by 15%.

P-60

CHIRAL SEPARATION OF ALPHA-HYDROXY ACIDS BY CAPILLARY ELECTROCHROMATOGRAPHY USING RISTOCETIN A AS CHIRAL SELECTOR

Martin G. Schmid, Julia Koidl, Iris Mühlhauser, Gerald Gübitz

Institute of Pharmaceutical Sciences, Dept. of Pharmaceutical Chemistry, Karl-Franzens-University, Universitätsplatz 1, A-8010 Graz, Austria martin.schmid@uni-graz.at

This work deals with the preparation of fritless particle-loaded capillaries for chiral separations by capillary electrochromatography. Silica particles containing a chemically bonded chiral selector are suspended in a methacrylamide monomer solution, which is *in-situ* polymerized in the capillary. Thus a particle-loaded monolith is formed containing the chiral selector embedded in a non-chiral continuous bed. Thereby the need of preparing frits is circumvented. Such monoliths are inexpensive and easy to prepare. Ristocetin A bonded to 3 µm silica particles was used as chiral selector. This approach was applied to the enantiomeric separation of chiral hydroxy acids. Since hydroxy acids migrate toward the anode, a cationic charge providing agent was copolymerized with the matrix. This served to reverse the direction of the EOF.

P-61

DETERMINATION OF TWO NEW ANTI-CANCER AGENTS IN PLASMA VIA A DOUBLE-CARTRIDGE-SPE-HPLC-METHOD

Margot Schober[1], Veronika Matzi[2], Alfred Maier[2], Joachim Greilberger[3], Giuseppe Martano[1,4], Ralph Herwig[5], Heinz Juan[6], Reinhold Wintersteiger[1]

(1) Inst. of Pharmaceutical Sciences, Karl-Franzens-University of Graz, Universitätsplatz 1, 8010 Graz, Austria; margot.schober@uni-graz.at
(2) Dept. of Thoracic and Hyperbaric Surgery, Medical University of Graz, Austria
(3) Inst. of Physiological Chemistry, Medical University of Graz, Austria
(4) Dept. of Pharmaceutical Sciences, University of Modena, Italy
(5) Dept. of Urology, Medical University of Vienna, Austria
(6) Dept. of Biomedical Research, Medical University of Graz, Austria

A new combination of two compounds including alpha-ketoglutaric acid (KG) and hydroxymethylfurfural (HMF), administered as a drinking solution, has shown great promise in anti-cancer therapy. The two main active and synergistically working compounds act as potential cell damaging agents. The solution still stands in phase II clinical study but the prosperities relating to a reduction of undesirable side effects and an appreciable improvement of life give reason to hope that a new alternatively working targeted therapy could have been designed. KG occurs naturally in the human organism as an intermediate of the citric acid circle but beside this, it is a powerful and effective scavenger, acts as an angiogenesis-suppressor by influencing the HIF-pathway and makes furthermore an impact on the metabolism of the tumour cells. HMF on the other hand shows ultra-selective cytostatic activities which are mainly caused by its resonance system and offers synergistic effects with KG. A combination of both agents in the ratio 1:3 HMF to KG shows good and promising results against various cancer types without any noteworthy damages to normal cells under test conditions and is therefore an other alternative to conventionally used chemotherapy especially for last-stage patients.

For the determination of the low levels of the active ingredients in plasma and for controlling their time-dependent degradation and the formation of potential sideproducts a double-cartridge SPE-technique was applied for monitoring KG- and HMF-levels after derivatisation. Best results were obtained using a polymeric cartridge for HMF and an anion-exchange cartridge for KG. Both SPE-columns were conditioned separately with methanol and water, then connected by an adaptor cap and spiked plasma was pipetted on the double-cartridge-system and slowly passed through the columns. After washing with water, the columns were disconnected and eluted separately with KH_2PO_4-buffer and MeOH, respectively. The MeOH was evaporated under nitrogen and the residue dissolved in the KH_2PO_4-buffer-elution. With respect to a higher sensitivity dinitrophenylhydrazine-derivatives have been formed and separated by HPLC on a C_8-column. The results showed good reproducibility and a LOQ of 3 ng analyte on column could be obtained.

P-62

VALIDATED ANALYTICAL METHOD FOR DETERMINATION OF L-CYSTINE, N-ACETYL-L-CYSTEINE, L-CYSTEINE AND REARRANGEMENT PRODUCTS IN AMINO ACID SOLUTIONS BY LC/MS/MS

Maria Suppan[1], Christoph Magnes[1], Barbara Daum[2], Peter Stadler[2], Thomas R. Pieber[1], Frank M. Sinner[1]

(1) Joanneum Research, Inst. of Med. Technologies and Health Management, Auenbruggerplatz 20, A-8036 Graz
(2) Fresenius Kabi Austria GmbH, Hafnerstraße 36, A-8055 Graz

Thiol compounds are sensitive to oxidation to disulfides, particularly in neutral or alkaline solutions [1]. Mixtures of these compounds can thus form rearrangement and by-products. Pharmaceutical amino acid solutions might contain more than one thiol amino acid (e.g. L-cystine, N-acetyl-L-cysteine). The aim of this project was to develop a method for the simultaneous quantitative determination of L-cystine, N-acetyl-L-cysteine and L-cysteine. An additional requirement of the method was that it should enable the semi-quantitative determination of three rearrangement products (N,N-diacetyl-cystine, N,S-diacetyl-cysteine, N-acetyl-cystine) in pharmaceutic amino acid solutions to allow the stability of the thiol compounds to be investigated.

The analytical method involves chromatographic separation followed by mass spectrometric detection in the positive ESI mode using selected reaction monitoring (SRM) scans. To maximise precision and accuracy, stable isotope-labelled internal standards were used. All experiments were carried out on a Dionex Summit HPLC System coupled to a Quantum TSQ Ultra AM (Thermo Finnigan). The stationary phase was a ZIC-HILIC column (2.1mm x 150mm, 3.5µm). The elution solvent A was 0.1% formic acid in acetonitrile and solvent B was 0.1% aqueous formic acid. Separation was performed by gradient elution (10 to 90 % B over 5 min, hold for 3 min, return to 10 % B over 0.1min and equilibrate for 9min). After a dilution step and addition of internal standards the samples were directly injected into the HPLC system. The method was validated according to ICH guidelines (stability, linearity, specificity, precision and accuracy).

The HILIC conditions permitted the separation of the hydrophilic compounds without the requirement for a derivatisation step. The optimized gradient elution allowed the separation of all interfering compounds in pharmaceutical amino acid solutions. The specificity was tested in the presence of 21 different amino acids. The validation procedure using L-cystine, N-acetyl-L-cysteine and L-cysteine quantification demonstrated the excellent quality of the LC/MS/MS method. Accuracies were between 97 and 103% and precisions were less than 4% in the range of 80 to 120% of the nominal concentrations in the investigated amino acid solutions. Advantages of this method are the simple sample preparation procedure and the rapid separation (17 minutes to determine five different compounds with high selectivity due to MS2 scans).

Stability studies of mixtures of L-cystine and N-acetyl-L-cysteine indicated the strong impact of the pH of the solution. After a few minutes, we were able to detect the byproducts N,N-diacetyl-cystine, N,S-diacetyl-cysteine and L-cysteine in neutral solutions. In acidic solutions, in contrast, no rearrangement products were found.

Reference [1] B. Toussaint, Ch. Pitti, B. Streel, A. Ceccato, Ph. Hubert, J. Crommen, Journal of Chromatography A, 896 (2000) 191-199

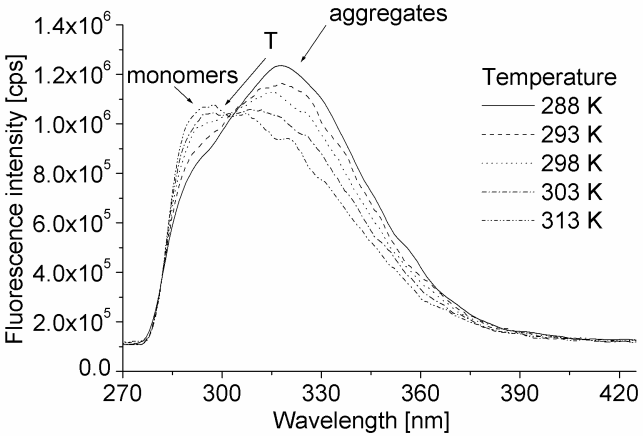
P-63

INCREASING THE WATER SOLUBILITY OF *O*-HYDROXY-ACETOPHENONE BY HYDROTROPIC *P*-TOLUENE SULFONATE

Kornélia Szabó[1], Géza Nagy[1], László Kollár[2], Sándor Kunsági-Máté[1]

(1) Department of General and Physical Chemistry, University of Pécs, Ifjúság 6, H-7624 Pécs, Hungary, kornelia@gamma.ttk.pte.hu,
(2) Department of Inorganic Chemistry, University of Pécs, Ifjúság 6, Pécs, H-7624, Hungary

Hydrotropes are a diversified class of molecules first described by Neuberg [1] almost a century ago. These compounds can be characterized by an amphiphilic molecular structure and by the ability to increase the solubility of sparingly soluble organic molecules in water, often by several orders of magnitude. Although they are widely used in several industrial applications, only little is known about their association thermodynamics.



In the present work the thermodynamics of molecular association of sodium *para*-toluene sulfonate (NaPTS) hydrotrope and *ortho*-hydroxy-acetophenone (*o*-HAP) mole-cules was studied by photo-luminescence (PL) measure-ments and quantum-chemical investigations. Significant changes of PL spectra with increasing the hydrotrope concentration is observed, which property reflects the molecular association [2]. Using this property we were able to determine the minimal hydrotrope concentration (MHC) and the stoichiometry of the aggregates. Evaluating by the van't Hoff theory the temperature dependence of the intensity of PL peaks associated to the monomer or multimer forms of NaPTS (see Figure), the Gibbs free energy, enthalpy and entropy changes of formation of multimers were determined simultaneously [3]. Quantum-chemical investigations showed that the association of NaPTS and *o*-HAP is preferably based on π-π stacking which however is moderated by dipole-dipole interactions in agreement with earlier results [4]. The analysis of partition functions of vibration and rotation movements of interacted molecules shows that the entropy changes observed experimentally are probably due to the inhibited molecular rotation of the associated species.

References

[1] Neuberg, C., *Journal of Chemical Society* **110** (1916) 555.
[2] Neumann, M.G., Schmitt, C.C., Prieto, K.R., Goi, B.E. *Journal of Colloid and Interface Science* **315** (2007) 810–813.
[3] Kunsági-Máté, S., Szabó, K., Desbat, B., Brunnel, J.L., Bitter, I., Kollár, L.: *Journal of Physical Chem.* **111** (2007) 7218-7223.
[4] Koparkar, Y.P., Gaikar, V.G. *J. Chem. Eng. Data* **49** (2004) 800-803.

P-64

DISSECTING THE MECHANOSENSOR FUNCTIONS OF TITIN

Dávid Szatmári, Tamás Huber, Viktória Németh, Veronika Kollár, László Grama, Miklós Kellermayer

University of Pécs, Medical School, Department of Biophysics

Titin is a giant protein strategically localized in the sarcomere for sensing the mechanical state of muscle. Although the amount of indirect experimental evidence supporting titin's mechanosensory function is on the rise, we know very little about the molecular mechanisms by which stretch and contractility information is converted into biomechanical signals. Our aim is to investigate the mechanisms by which force-driven structural changes in titin may lead to the generation of transmissible signals. We hypothesize that titin carries out spatially distributed, differentiated tasks: sustained-force sensing in the Z-line, mechanical-transient sensing in the I-band, and structural-shift sensing in the M-line.

We studied three different elements of titin located in the Z-line, the I-band and the M-line, respectively. Each fragment was cloned from a human soleus muscle cDNA library, expressed in *E. coli*, and purified using metal-affinity chromatography.

Z1Z2, a fragment comprising two Ig domains in titin's N-terminal region, interacts with telethonin, a protein that anchors titin to the Z-line. This complex is involved in signalling processes that may regulate muscle development and degradation. We examine the stability of Z1Z2 and the Z1Z2-telethonin complex by using atomic force microscopy (AFM) and optical tweezers.

In the M-band, titin contains a catalytic domain, the titin kinase, that is thought to become activated by mechanical forces. We expressed the titin kinase together with its neighbouring domains. To characterize the mechanism of its force-induced activation, we stretch the fragment with optical tweezers, and use a synchronized total internal reflection fluorescence microscopy and atomic force microscopy (TIRF-AFM) system to to explore the correlation between ATP-binding and mechanical events..

Under physiological conditions the major source of titin's extensibility is PEVK, a domain located in the I-band region of titin. PEVK, which comprises two major types of motifs, PPAK and polyE, is thought to be an intrinsically disordered domain. We explored the structural dynamics of fragments rich in either of the motifs by *Trp* fluorescence quenching and spectroscopic analysis of rhodamine (TMRIA)-labeled fragments. Rhodamine dimers were found to dissociate during chemical denaturation of the TMRIA-labeled fragments, indicating the presence of dynamic intramolecular interactions. The polyE rich PEVK motifs displayed calcium-dependent structural changes, suggesting a modulation of fragment structure by calcium binding.

Our results and preliminary observations suggest that titin may indeed exhibit various mechanosensor functions that contribute to a highy differentiated monitoring of muscle's complex mechanical environment.

P-65

POLYLUMINOL BASED BIOSENSOR FOR H_2O_2

Mónika Szili, Géza Nagy, Barna Kovács

Department of General and Physical Chemistry, Faculty of Natural Sciences, University of Pécs, Ifjusag utja 6., 7624 Pécs, Hungary,
kovacs1@gamma.ttk.pte.hu

Chemilumiscent reactions of luminol are well known in the analytical as well as the clinical chemistry. Luminol can be polymerized by electrochemical way [1], the resulting polyluminol (PL) also emits light in the present of oxidizing agents. The main advantage of PL is that the solid state sensor has longer shelf lifetime, than the luminol solutions.
In the present work PL was prepared by cyclic voltammetry (50 cycles, 100 mV/s) onto indium-tin-oxide (ITO) coated microscope glass. The PL surface was covered by hydrogel using spin-coating machine to make it hydrophilic. A PVC sheet containing 6 mm diameter holes was glued onto the top of the hydrogel layer to form 21 individual sample cells. Horseradish peroxidase (HRP) was used as catalyst in the luminol-hydrogen-peroxide reaction [2], and it was immobilized in alginate gel prepared in the cells. The alginate gel was prepared from 2 % alginate solution, the cross linking was made with 0,05M $CaCl_2$ solution. Since the signal was directly proportional to the enzyme activity, measurements were made at constant (108Unit/ml) HRP concentration. Effects of buffer composition and pH, enhancer, concentration, enzyme activity were studied. Signals obtained by using dissolved and immobilized enzymes were compared. The optimal pHs were 7.6 and 7.2 in solution and by using immobilized enzyme, respectively. The highest luminescence intensity was observed in carbonate buffer in solution, but because it changes its pH rapidly, measurements were done in phosphate buffer. Two enhancers, para-iodophenol (PIP) and phenylimidazole (PHI) were tested and compared. In the presence of PIP a three times higher signal was measured, while PHI caused a five fold increase in the luminescence intensity. The optimal concentration of PHI was 2 mmol/l. Hydrogen peroxide was detected from gas in a flow through cell. The top of the sample compartment was closed with a reflecting alumina sheet before this measurement. The gas in- and outlet were on the opposite sides of the cell. Nitrogen gas was bubbled in buffered H_2O_2 solutions having different concentrations, and it was guided through the cell. The measured peroxide concentration was $1{,}5*10^{-5}$ M in the gas phase.

References

[1] Shen-Ming Chen, Kuo-Chiang Lin, J. Electroanal. Chem. 523 (2002) 93-105.
[2] Yannis Dotsikas, Yannis L. Loukas, Anal. Chim. Acta, 509 (2004) 103-109.

P-66

OPTIMIZATION OF EXTRACTION METHODS FOR ANTIOXIDANTS FROM POLYOLEFINS

Martin Trötzmüller, Ernst P. Lankmayr

Graz University of Technology, Institute of Analytical Chemistry and Radiochemistry, Technikerstraße 4, 8010 Graz, Austria
m.troetzmueller@gmail.com

Polyolefins are the most widely used thermoplastic polymers and are employed in an extremely wide range of applications. Commercial polyolefins need the addition of suitable amounts of antioxidants to prevent their degradation both during processing and their lifetime and are designed to get best performances in their specific end-use applications. Therefore, one important issue for polymer production is the quantitative determination of all antioxidants contained in the individual polymers.
Owing to their physical properties, an analysis of the polymer additives such as antioxidants can be complicated considering the necessary aspects for the quantitative and reproducible extraction of the additives from the polymer matrix. Traditional liquid solvent polymer extraction methods such as Soxhlet- or batch-extraction are time-consuming and inefficient. Microwave heating is gaining favour for the extraction of antioxidants from polymers because of the relatively short sample preparation times. In addition, sample throughput is improved since several samples can be simultaneously extracted. Microwave-assisted extraction (MAE) may also minimize the quantities of organic solvents used for the extraction. Therefore, this kind of extraction is a simple and straightforward technique.
In the present study mainly MAE was investigated for the extraction of the antioxidants BHT, Irganox 1010, Ultranox 626, Irgafos 168 and Irganox 1076 from polyethylene (PE) and polypropylene (PP) samples [1]. The extraction procedure was first screened according to a complete factorial design for statistically significant parameters. Thereafter, the identified parameters were optimized via a Box-Behnken design and computation of a response hypersurface. Therefore it is possible to define the optimum parameters for MAE of the antioxidants from pelletized polyolefins. Additionally, a comparison between this approach and the traditional batch-extraction with chloroform as solvent was performed. The extractions were carried out from pelletized as well as ground polymer samples and the results obtained were compared. Furthermore, accelerated solvent extraction (ASE) was examined for its extraction-efficiency and compared with MAE. The final determination of the antioxidants was performed directly by reversed phase HPLC-UV without the need of any concentration steps of the extracts.

Reference

[1] Martin Trötzmüller, 2006: Extraction of antioxidants from polyolefins and determination by HPLC-UV; Diploma thesis, Institute for Analytical Chemistry and Radiochemistry, Graz University of Technology, Austria

P-67

FEATURES AND ORIGINS OF COMMON CHEMICAL BACKGROUND IONS IN ESI-HPLC-MS/MS

Martin Trötzmüller, Xinghua Guo and Ernst P. Lankmayr

Graz University of Technology, Institute of Analytical Chemistry and Radiochemistry, Technikerstraße 4, 8010 Graz, Austria
m.troetzmueller@gmail.com

The interference of chemical background ions has been a problem since the inception of liquid chromatography-mass spectrometry (LC-MS). Chemical ionic noise refers to charged species derived from cluster ions from LC mobile phase and contamination in mobile phase, tubing materials and even the laboratory air [1]. It imposes a major obstacle on the sensitivity of atmospheric pressure ionization (API) LC-MS. In our previous study, the knowledge about the structures of chemical noise has led to the successful development of a noise reduction technique [2].
In this presentation we have continued the investigation on typical negative backgrounds. It was carried out by studying the products and precursors of the major background ions to find their structure relationship using tandem mass spectrometry. Various typical LC eluents with different compositions and additives such as ammonium formiate/formic acid or ammonium acetate/acetic acid were investigated. Similarly to previous observation [1], two groups of noise ions are concluded, that are mainly the chemical backgrounds of cluster ions from solvents and additives, accompanied by some minor contribution from typical individuals contaminants (e.g. in additives and degradation products from tubing, impurities in mobile phase, etc.). Furthermore, some clusters are also from the solvation of some contaminants.
The obtained information from this work can help to understand the formation of negative chemical noise in LC-MS and can also be used to develop methods for noise reduction.

References:
[1] Guo X., Bruins A.P. and Covey T.: Characterization of typical chemical background interferences in atmospheric pressure ionization liquid chromatography-mass spectrometry, *Rapid Communications in Mass Spectrometry*, 20, 3145–3150 (2006).
[2] Guo X., Bruins A.P. and Covey T.: Method to reduce chemical background interference in atmospheric pressure ionization liquid chromatography-mass spectrometry (API LC-MS) using exclusive reactions with the chemical reagent dimethyl disulfide, *Analytical Chemistry* 79(11), 4013-4021 (2007).

P-68

ENANTIOSELECTIVE CHROMATOGRAPHIC AND CAPILLARY ELECTROPHORETIC DETERMINATION OF THE BETA-2-SYMPATHOMIMETIC FENOTEROL FOR PHARMAKOKINETIC STUDIES

Thomas Ulrich[1], Gerd-Joachim Krauss[1], Dirk Wesenberg[1], Martin G.Schmid[2], Gerald Gübitz[2]

(1)Institute of Biochemistry, Dept. of Biochemistry/Biotechnology, Martin-Luther University Halle-Wittenberg, Kurt Mothes Straße 3, D-06120 Halle/Saale, BRD
(2) Institute of Pharmaceutical Sciences, Dept. of Pharmaceutical Chemistry, Karl-Franzens University, Universitätsplatz 1, A-8010 Graz, Austria, guebitz@uni-graz.at

The development of methods for the determination of fenoterol by chiral HPLC and CE is described. For HPLC separation precolumn fluorescence derivatization with naphthyl isocyanate was applied. The resulting urea derivatives were separated on a cellulose tris (3,5-dimethylphenylcarbamate) coated silica gel column employing a column switching procedure for sample pretreatment. Detection was carried out fluorimetrically with a detection limit in the low ng/ml range. The method was applied to rat heart perfusates comparing liquid-liquid extraction and solid phase extraction approaches. As an alternative a CE method was checked using different ß-CD derivatives as chiral selectors. Although with this simple technique good separations were obtained, the sensitivity was not sufficient for pharmakokinetic studies.

P-69

MODELLING OF OVERLOADED BAND PROFILES OF ORGANIC MODIFIERS ON C-18 RPLC COLUMN

Péter Vajda[1], Szymon Bocian[2], Bogusław Buszewski[2], Attila Felinger[1]

[1] Department of Analytical and Environmental Chemistry, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary
[2]Department of Environmental Chemistry & Bioanalytics, Faculty of Chemistry Nicolaus Copernicus University, 7 Gagarin Street, PL-87100 Torun, Poland

Very important for understanding chromatographic separation processes, and competitive adsorption of solvents from the mobile phase and the solute, because these processes govern the adsorption of the compound we want to separate of purify. The organic solvent adsorption may be determined using numerical method and it is useful also to estimate the surface heterogeneity. Two commonly used organic modifiers (THF, 2-propanol) were chosen for these measurements.

The breaktrough curves of THF and 2-propanol from pure water were calculated using the equilibrum-dispersive model (ED) of chromatography. This model assumes instantaneous equilibrum between the two phases of the chromatographic system, and a finite column efficiency originating from an apparent axial dispersion coefficient, that accounts for axial dispersion and for the mass transfer resistances in the chromatographic bed. This model is widely used to describe the overloaded band profiles of several molecules with low molecular masses.

The inverse method (IM) was used to derive the best values of the isotherm parameters from overloaded band profiles. To use this method an isotherm model and initial adsorption parameters needed. From the shape of the band profiles we can assume that the adsorption behavior of the two studied compound can be describe with an isotherm model which belongs to the “Langmuir-family”. The best model was selected using the residual sum of squares (RSS), for each selected isotherm model comparison the numerically calculated and the measured band profile. The best fitted model was used to estimate the heterogeneity of the adsorbent surface.

This work was sponsored by Ceepus II scholarship CII-PL-0004-03-0708-PL-130-05/06, as well as by grants OTKA T48887 and OTKA-NKTH NI68863.

P-70

ANTIOXIDANT PROPERTIES OF HYDROXBENZOIC ACID DERIVATIVES

Beata Veliká, Ivan Kron

Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, PJ Šafárik University, Tr. SNP 1, 040 66 Košice, Slovakia, ivan.kron@upjs.sk

Salicylic acid (*o*-hydroxybenzoic aid) is one of the oldest drug used in medicine, either free or in the ester form with acetic acid [1]. Besides antipyretic, antirheumatic and analgesic properties, it also shows antioxidant activity due to the presence of a phenolic hydroxyl group. The position of the hydroxyl group could be important for increasing antioxidant activity. Therefore *o*-, *m*-, and *p*-hydroxy-substituted benzoic acids were tested for their ability to react with superoxide radicals generated by methionine-riboflavin generator [2].
Kinetic analysis showed significant differences between the investigated derivatives. The best antioxidant properties was recorded for the *m*-hydroxybenzoic acid, which is in accordance for antioxidant activities determined with Trolox [3].
The authors discuss the structure-antioxidant activity relationship related to kinetic of the electron transfer within molecules of hydroxybenzoic acids.

References

[1] Collier, H.O.J.: The story of aspirin, in *Discoveries in Pharmacology*, (Eds. Parnham M.J. and Bruinvels J.), Elsevier 1987, New York, pp. 147-185
[2] Beauchamp, C., Fridovich, I., *Anal. Biochem.* **44** (1971) 276-287.
[3] Rice-Evans, C.A., Miller, N.J., Paganga, G., *Free Radic. Biol. Med.* 20 (1996) 933-956.

P-71

CONTEMPORARY LIGNIN ANALYTICS BY NMR-SPECTROSCOPY

Hedda K. Weber[1], Moritz Leschinsky[1], Petra Wollboldt[1], Gerhard Zuckerstätter[1], H.Sixta[2]

(1) Kompetenzzentrum Holz GmbH, St.-Peter-Str. 25, 4021 Linz, Austria
(2) Lenzing AG Pulp Research, Werkstraße 1, 4860 Lenzing, Austria
h.weber@lenzing.com

The first step in the utilisation of wood in the pulp and paper industries is its decomposition mostly employing the sulphite cooking and the prehydrolysis Kraft cooking, respectively. Although these processes date back to the penultimate century there are lots of unsolved problems. When modifying the process often the lignin is altered in such way that it becomes insoluble for the subsequent process steps. In order to understand the reasons for these effects an extensive toolbox of methods to analyse lignin and its degradation products is required. Since the native lignin is a very complex molecule the pulping experiments are also carried out with model compounds representing the most common structural units of the lignin macromolecule. A most helpful method in characterising the lignin, its degradation products and the model compounds is NMR-spectroscopy and, in particular, two-dimensional NMR spectroscopy.

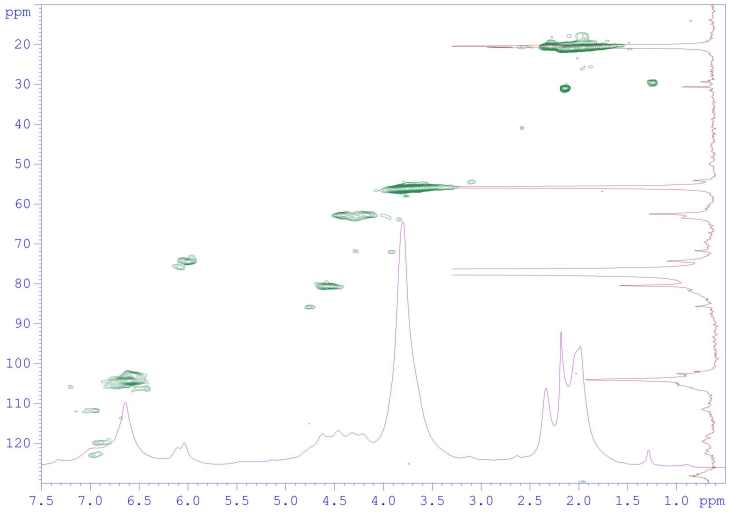


Figure: 2D-NMR spectrum of DWL

As an example we present the results from the prehydrolysis experiments. The lignin was isolated after the water prehydrolysis step and compared to the lignin isolated from *Eucalyptus globulus* wood as MWL. Additionally, lignin fractions that precipitated from the prehydrolysate under various conditions were investigated. The results were related to a study comprising five dimeric lignin model compounds. Whereas complete cleavage of phenolic β-O-4 models led to a complex mixture of reaction products and β-1 structures were transformed into stilbenes, the models for non-phenolic β-O-4 and for β-5 moieties remained stable under those reaction conditions.

P-72

PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS OF ROSE HIP POWDERS

Ute Widowitz[1], E.M. Wenzig[1], S. Chrubasik[2], E. Knauder[1], F. Bucar[1], R. Bauer[1]

(1) *Institute of Pharmaceutical Sciences, Dept. Pharmacognosy, University of Graz, Universitaetsplatz 4, 8010 Graz, Austria, email: ute.widowitz@uni-graz.at*
(2) Institute of Forensic medicine, University of Freiburg i.Br., Albertstr. 9, 79104 Freiburg i.Br., Germany

Rosae pseudofructus (Rose hip) is not only used for the prevention and cure of common cold, as a diuretic agent and as vitamin C source, but also for the treatment of rheumatic diseases.
The aim of this study was to compare two different powders of rose hips (*Rosae pseudofructus cum* (RCF) and *sine fructibus* (RSF) concerning their constituents and their pharmacological effects *in vitro* (inhibition of COX1/2, 5-LOX, and radical scavenging activity).
The comparison of the compounds of RSF and RCF showed that they differ in the amount of fatty acids and triterpene acids. It turned out that they also differ in terms COX1/2 and 5-LOX inhibition.

	IC50 COX1(µg/ml)		IC50 COX2 (µg/ml)		IC50 5-LOX (µg/ml)		% Betulinic acid ± SD		% Oleanolic acid ± SD		% Ursolic acid ± SD	
Extrakt	RSF	RCF	RSF	RCF	RSF	RCF	RSF	RCF	RSF	RCF	RSF	RCF
n-hexane	22,9	>125	10,4	48,1	25,4	92,2	4,09± 0,07	0,46± 0,00	0,76± 0,01	_	0,29± 0,01	_
dichloro-methane	35,4	>125	29,2	>125	41,5	74,2	7,88± 0,12	1,8± 0,03	1,45± 0,07	1,17± 0,01	1,02± 0,04	1,34±

Table 1: Comparison of lipohilic RSF and RCF extracts concerning their IC50 values and contents of triterpene acids

P-73

THIN LAYER CHROMATOGRAPHIC ANALYSIS OF CAROTENOIDS

Alam Zeb, Michael Murkovic

Institute for Food Chemistry and Technolog, Technische Universität Graz (TUG)
Petersgasse 12/2; A-8010 Graz, Austria
a.zeb@student.tugraz.at

Thin-layer chromatography (TLC) is a simple, rapid and inexpensive method for the separation, identification and visual semi-quantification of a wide variety of food materials. However little attention has been given regarding carotenoids analysis by TLC. The present study reveals the uses of TLC analysis of different carotenoids and the importance for investigation of the antioxidant potential of different food products.

P-74

OPTIMIZATION OF CAPILLARY ISOELECTRIC FOCUSING FOR THE ANALYSIS OF AMINO ACID DERIVATIVES

Lubomir Georgiev, Tamara Pajpanova, Ferenc Kilár

Neofit Rilski University, Blagoevgrad, Bulgaria
University of Pécs, Pécs, Hungary

Isoelectric focusing (IEF) is a powerful technique used for the separation of protein mixtures as well as amino acids, based on differences in isoelectric points (pI), likewise determination of the same. Isoelectric focusing (IEF) is a well-known separation technique, introduced for biochemical separations. In this work we are presenting a method for routine analysis, for the system we have, for determination of pI of amino acids and derivatives which will be applicable and for proteins, even more suitable for them. Capillary IEF is an electrophoretic technique for separation of amphoteric compounds in a pH gradient which is formed by ampholytes in an electric field using coated or uncoated capillaries. In depends of the capillary there is a difference in the conditions for mobilization of the gradient in order to move the analytes toward the detection point [1-4]. In this case we use the set-up reported previously by Kilar and al. [5]. In briefly this set-up includes separate injection of the ampholytes and the sample how is presented in fig.1. In previous CIEF set-ups, sample and ampholytes were introduced in a mixture, but the possible interactions between them should not be excluded, which may disturb the analysis.

This work was supported by the Ceepus CII-HU-010 Network and the OTKA-NKTH NI68863 grant.

P-75

ELASTICITY AND FORCE-INDUCED STRUCTURAL TRANSITIONS OF INDIVIDUAL DESMIN INTERMEDIATE FILAMENTS

Balázs Kiss, Árpád Karsai, Miklós S.Z. Kellermayer

Department of Biophysics, University of Pécs, Faculty of Medicine, Szigeti út 12. Pécs H-7624 Hungary

Desmin filaments form the intermediate filament system in muscle cells and are thought to be important in determining their mechanical integrity and elasticity. The molecular basis of desmin's elasticity is not fully understood. In the present work we mechanically manipulated desmin filaments polymerized from purified monomers, by using single-molecule atomic force microscopy (AFM). Because phosphate-treatment is expected to cause structural disruption, we analyzed the effect of Na-phosphate on the morphology and force response of individual desmin filaments.

Desmin, purified from chicken gizzard, was polymerized by the addition of either $MgCl_2$ or NaCl. For mechanical manipulation desmin filaments, adsorbed to mica or silanized glass surface, were captured with the tip of a flexible AFM cantilever. The filaments were then stretched by moving the cantilever away from the surface. Mechanically manipulated desmin displayed complex force responses. We identified four fundamental types of mechanical behavior: a) initial transition, b) force plateau c) plateau bumps and d) non-linear elasticity.

a) The initial transition trace was the most frequently observed force pattern characterized by two discrete 20-60 pN force steps. This may correspond to unbinding and removal of individual coiled-coil desmin dimers from the filament surface.

b) Force plateaus are characterized by constant force as a function of extension and resemble polymer desorption processes by protofilaments longer than 60 nm.

c) Plateau bumps were superimposed on force plateaus in 16-nm steps. Conceivably, these force transitions appear as a result of unzipping or peeling protofilaments away from the surface of the desmin filament.

d) Non-linear force curves often followed in tandem to form a sawtooth pattern. The non-linear curves were fitted with the wormlike chain model of entropic elasticity to obtain the persistence length (measure of bending rigidity) of the mechanically manipulated chains. The mean persistence length acquired from force measurement experiments was ~0.4 nm, which is far below previous measurements for intermediate filaments (~1 µm).

Considering that the persistence length was similar to that of unfolded protein molecules (e.g., mechanically unfolded titin), it is conceivable that the non-linear force curves reflect the behavior of unfolded desmin monomers/protofilaments. To independently assess the entropic elasticity of unperturbed desmin, we analyzed the shape fluctuations of surface-adsorbed filaments. Based on this shape analysis the persistence length of desmin filaments is ~0.45 µm, and the calculated Young modulus is 3.7 MPa. The obtained quantitative measures of desmin elasticity may provide a basis for estimating desmin-associated mechanical features at the muscle fiber level. In phosphate-treated samples we detected a decrease in the numbers of parallel attached subfilamental units which refers to the unraveling of the filamentous structure. Based on the decresae of

plateau length, plateau bump spacing and maximal nonlinear force responses we hypothesize the role of electrostatic interactions in these structural changes.
In summary, a complex set of coupled intrafibrillar structural changes are evoked in desmin intermediate filaments under mechanical stress.

P-76

SIMPLE TEST FOR THE SELECTIVITY OF THE ARTIFICIAL GEL ANTIBODIES BY FLUOROMETRY

Nasim Ghasemzadeh[1,2], Fred Nyberg[2], Stellan Hjertén[1]

[1]Department of Biochemistry and Organic Chemistry, Biomedical Center, Box 576, SE-751 23 Uppsala.
[2]Department of Pharmaceutical Biosciences, Biological Research on Drug Dependence, Biomedical Center, Box 591, SE-751 24 Uppsala. E-mail: Nasim.G@farmbio.uu.se

Artificial gel antibodies can be synthesized by a universal method against biopolymers, for instance proteins, and bioparticles, for instance viruses and cells. The stability of these antibodies are much higher than for the native protein antibodies.
We have designed a calibration curve, based on the light absorption of the stained protein, adsorbed to gel antibodies. The calibration curve is used for rapid determination of the concentration of the "antigen" in a sample solution, for instance blood serum.
One potential application of the artificial gel antibodies is to "fish out" a biomarker for a specific disease from serum for diagnosis and prognosis of this disease. The calibration curve we presented can be used for the determination of the concentration of the protein biomarker down to 20 µg protein/ml in the body fluid.
We have also developed a technique based on fluorescence, which gives a much high sensitivity for detection of the antigen selectively adsorbed to artificial gel antibodies. In these studies we used fluoroprobes attached to the ″antigens″ in combination with fluorescence imaging to test the selectivity of artificial gel antibodies. These artificial gel antibodies show high selectivity and are easy to prepare.
Fluorescence measurements in the scanning mode have the advantage that each gel granule becomes visible. Other advantages are that an only a small amount of the sample is required for the detection and that the time for an analysis is short.