# ABSTRACTS

# LECTURES

# L-1 - L-21

**L-1**

Stellan Hjertén, Ivett Bacskay, Anikó Takátsy, Torbjörn Svensk, Marie-Christine Hjertén, Ákos Végvári, András Ballagi-Pordány, Anders Elfwing, Ferenc Kilár

# ARTIFICIAL ANTIBODIES

*University of Uppsala and University of Pécs*

In conventional electrophoresis and chromatography one strives for high plate numbers in order to allow separation of a substance of interest from other substances in the sample, whereas in affinity methods plate numbers have no significance. Among the latter methods, those based on native antibodies raised in animals have been of great importance and it has been generally assumed that their selectivity cannot be surpassed by artificial antibodies synthesized by purely chemical methods. This idea is based on feelings rather than facts and has certainly hampered the progress of the molecular imprinting technique. Let us therefore consider both theoretically and experimentally the veracity of this "dogma".

The experiments we will present are based on molecular imprinting of proteins, and bioparticles, such as viruses and bacteria, a mechanism which was proposed by Pauling in the forties for the formation of *native* antibodies. The first successful laboratory synthesis of *artificial* antibodies against proteins based on this hypothesis was not reported until 1996 (1) . For low-molecular-weight compounds the molecular-imprinting approach was introduced independently in 1972 by Wulf and Klotz. The time gap of about a quarter of a decade reflects the difficulties to find appropriate experimental conditions to get a selective imprint of large molecules, such as proteins. Therefore, we will discuss important features of the synthesis method as well as the issue of whether the artificial antibodies may have a higher selectivity than the native ones and then show experiments which do not contradict such a highly attractive property.

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**L-2**

Martin G. Schmid, Nina Grobuschek, Christina Freigassner, Maria Hölbling, Andrea Klostius, Verena Pessenhofer and Gerald Gübitz

# CHIRAL SEPARATION OF DIPEPTIDES BY MICRO-HPLC AND CAPILLARY ELECTROCHROMATOGRAPHY

*Institute of Pharmaceutical Chemistry and Pharmaceutical Technology, Karl-Franzens University Graz, A-8010 Graz, Austria*

Dipeptides are basic and essential components in biological systems either as individual compounds or as constituents of proteins. Since the presence of a D-amino acid iin a peptide may result in different pharmacological activity, enantioseparation of amino acids and peptides is of big importance. There are different approaches to perform enantioseparation of dipeptides. In this study, the use of µ-HPLC is compared with CEC. Macrocyclic antibiotics were used as chiral selectors and immobilized on the stationary phase. Furthermore, in CEC, preparation of capillaries packed with silica chiral stationary phases (CSPs) is a sophisticated procedure, since high pressure has to be applied for packing and frits have to be prepared by sintering a zone of a silica based packing. A new approach to fix a silica chiral stationary phase in a capillary is also shown in this work: Silica particles containing a chiral selector are suspended in a monomer solution, which is sucked into the capillary followed by *in-situ* polymerization. Thus, particles are embedded in a non-chiral continuous bed.

This kind of CSP is inexpensive and easy to prepare and circumvents the use of frits. Separation ability of a continuous bed containing antibiotics as a chiral stationary phase was compared to a capillary packed with silica particles of the same selector. These approaches were successfully applied to the enantioseparation of amino acids, dipeptides and drugs. This work focuses on the application of these techniques for glycyl- and diastereomeric dipeptides. As chiral selectors, teicoplanin, teicoplanin aglycone [1, 2] and ristocetin A were used.

[1] Enantioseparation of dipeptides by capillary electrochromatography on a teicoplanin aglycone chiral stationary phase
M. G. Schmid, N. Grobuschek, V. Pessenhofer, A. Klostius, G. Gübitz, J. Chromatogr. A 990 **2003** 83-90

[2] Chiral resolution of diastereomeric di- and tripeptides on a teicoplanin aglycone phase by capillary electrochromatography
M. G. Schmid, N. Grobuschek, V. Pessenhofer, A. Klostius, G. Gübitz, Electrophoresis, 25 (15) **2003**, 2543-2549

**L-3**

Ákos Végvári, Stellan Hjertén

# STABLE HOMOGENEOUS GELS FOR ELECTROPHORETIC SEPARATIONS OF PROTEINS IN CAPILLARIES AND GLASS TUBES

*University of Pécs and University of Uppsala*

Homogeneous gels are well known and widely used in different formats for electrophoretic separation of a variety of classes of substances. We have developed a polyacrylamide-based gel cross-linked with a modified β-cyclodextrin, which can be used not only for capillary electrophoretic (CE) but also for electrochromatographic (CEC) applications. These multi-purpose gels have long lifetime and low UV absorbance, permitting on-line detection. Additionally, it is easy to prepare them and they can be attached to the capillary wall covalently.

The electrophoretic gels combine the famous high resolution of acrylamide gels with simple preparation and handling.

The separation of proteins is often performed in slab gels arranged in horizontal or vertical electrophoresis apparatus. Using such gels in capillary is more beneficial due to the advantages of this format. It is possible to detect the separated zones directly over the capillary in UV-light; these gels can be repeatedly used without significant changes in performance and the pore-size can be set

We will exemplify the above statements with CE experiments in capillaries and glass rods of different diameter for comparison. For this later format colored protein molecules (hemoglobin and phycoerythrine) were chosen, of which differences in electrophoretic properties and size are salient.

**L-4**

Kevin A. Francesconi

# MASS SPECTROMETRIC METHODS FOR DETERMINING ARSENIC COMPOUNDS

*Institute of Chemistry-Analytical Chemistry, Karl-Franzens University, Universitaetsplatz 1, 8010 Graz, Austria*

Although the element arsenic is best known for the toxic properties of some of its inorganic acids, it is also found in many naturally-occurring, and mostly non-toxic, organic forms. Studies on the biogeochemistry and toxicology of arsenic require analytical methods to individually determine these various chemical forms (or species) of arsenic. This general field of analytical chemistry is termed speciation analyses, and the determination of arsenic species constitutes an important part of this field.

Early methods of arsenic speciation analyses were restricted to As-rich samples or to those few arsenicals that could be readily derivatised to volatile analytes. The situation changed radically in the late 1980s, however, with the commercial introduction of inductively coupled plasma mass spectrometers (ICPMS). These instruments provided great sensitivity and element selectivity, and they could be readily coupled with a separation system, such as HPLC. The resulting technique HPLC/ICPMS was able to separate and reliably quantify mixtures of arsenic species present in environmental and biological samples, and thereby was able to provide data for assessment of the toxicological risks associated with arsenic. The development of soft ionisation sources (eg electrospray ionisation) for use with mass analysers and their application to arsenic species has lead to further developments in this field, especially in the identification of new arsenic compounds.

The developments in mass spectrometric techniques for determining arsenic compounds will be discussed and illustrated with examples of applications. Emphasis will be placed on the relevance (and non-relevance!) of arsenic speciation analyses in the environmental and human health areas. Future applications of mass spectrometry to this field will also be briefly discussed.

**L-5**

Frank M. Sinner, Christoph Magnes, Christina Gatschelhofer,

Michael. R. Buchmeiser, Thomas. R. Pieber

# MONOLITHIC STATIONARY PHASES: MONOLITHS PREPARED BY RING-OPEN METATHESIS POLYMERISATION (ROMP) FOR NANO-HPLC-MS

*Joanneum Research, Inst. of Med. Technologies and Health Management,*
*Auenbruggerplatz 20, A-8036 Graz*
*email: frank.sinner@joanneum.at*
*Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens-University,*
*Innrain 52a, A-6020 Innsbruck*

The preparation of monolithic supports for analytical science *e.g.* High Performance Liquid Chromatography (HPLC) has gained significant interest over the last years. These materials exhibit a number of advantages compared to particle packed columns *e.g.* lower back pressure combined with enhanced diffusion mass transport, less void volume and no further need for packing. This leads to very fast separations, due to high flow rates combined with enhanced mass transfer between the mobile phase and the monolithic surface. Furthermore, monoliths can easily be downscaled and prepared at low cost.[1]

In this contribution we report on straightforward and broadly applicable synthetic routes for the preparation of non functionalised [2] and "in-situ"-functionalised monolithic [3] supports. 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 the Grubbs-type initiator $Cl_2(PCy_3)_2Ru(=CHPh)$ in the presence of a mixture of toluene and 2-propanol. The resulting non functionalised separation media – which are polymerised in the confines of the separation molds (HPLC-columns and capillaries) - possess different microstructures depending on polymerisation parameters and monolith size.

Functionalised continuous rods were synthesised by one additional synthetic step that takes advantage of the living character of the ROMP-based copolymerisation. This "in-situ" derivatisation was achieved after the formation of the continuous rod by reacting the active, surface-bound initiator with functional, norborn-2-ene- and 7-oxanorborn-2-ene-based monomers.

This contribution will cover investigations concerning non-functionalised and functionalised monolithic separation media with inner diameter from 2 mm down to 75 µm. The effect of the polymerisation procedure on separation performance, on resulting monolithic structures, and on separation of peptides and proteins will be discussed.

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[2] F. M. Sinner, M. R. Buchmeiser, Angew.Chem. 2000, 112 1491-1494.
[3] B. Mayr, F. Sinner, M. Buchmeiser, J.Chromatogr.A 2000, 907 47-56.

**L-6**

Franz Bucar

# LC-MSn ANALYSIS OF FLAVONOIDS IN MEDICINAL PLANTS

*Institute of Pharmacognosy, University of Graz*
*Universitätsplatz 4/1, A-8010 Graz, Austria*

Flavonoids represent a diverse group of more than 4000 polyphenolic compounds with important impact on medicinal plant extracts due to their biological properties such as antioxidant, anti-inflammatory or cancer chemopreventive effects [1-3]. Flavonoids can be classified into major classes according to the oxidation level of their central C-ring: flavones/flavonols, flavanols and related procyanidins, anthocyanins and flavanones. Many structural variations exist within each class depending on hydroxylation, glycosidation and other substituents present which makes analysing crude plant extracts for their flavonoid pattern a challenge.

Results from LC-$MS^n$ analysis of crude extracts from leaves of different *Ribes sp*., which are rich in flavonols, with quadrupol ion trap mass analyser will be presented. By qualitative analysis using LC-ESI-MS besides a number of flavonol glycosides (including acylated flavonol glycosides), in leave extracts of two *Ribes sp.* myricetin 3-O-glucuronide, a flavonoid previously identified as strong anti-inflammatory principle in leave extracts of *Epilobium angustifolium* [4] could be found by. Quantification was carried out comparing SIM (selected ion monitoring) and SRM (selected reaction monitoring) modes and showed higher quantities of myricetin 3-O-glucuronide in *Ribes* than in *E. angustifolium* leaves.

Further examples of usefulness of LC-MS methods in flavonoid analysis concern C-glycosyl flavones and acylated flavonol glycosides. ESI-$MS^2$ and ESI-$MS^3$ spectra made differentiation between 6,8-di-C-glucosylflavone (vicenin-2) and vitexin 2´´-O-glucoside (2´´-O-glucosyl-8-C-glucosylflavone easily possible. Generally, for glycosides highest sensitivity was found in negative ESI-MS mode.

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[2] Middleton, E Jr. Effect of plant flavonoids on immune and inflammatory cell function. Advances in Experimental Medicine and Biology 1998, **439** (Flavonoids in the Living System): 175-182.

[3] Rice-Evans, CA, Miller, NJ, Paganga, G. Structure - antioxidant acitivity relationships of flavonoids and phenolic acids. *Free Radicals in Biology and Medicine* 1996, **20**: 933-56.

[4] Hiermann, A, Schramm, HW, Laufer, S. Anti-inflammatory activity of myricetin-3-O-β-D-glucuronide and related compounds. *Inflammation Research* 1998, **47**: 421-427.

**L-7**

Bernd Trathnigg

# CHARACTERIZATION OF NONIONIC SURFACTANTS BY DIFFERENT CHROMATOGRAPHIC TECHNIQUES

*Institute of Chemistry, Carl-Franzens-University, Heinrich str. 28., A-8010 Graz, Austria*

In the analysis of non-ionic surfactants different mechanisms can be applied, the suitability of which depends on the nature of the hydrophobic part, the oligomer distribution of the oxyethylene chain, and the architecture of the entrire molecule.

In special cases (pure starting material, no excessive side reactions), a full separation can be achieved in one chromatogram, the typical situation (technical starting material, side reactions not negligible) requires however a two-dimensional separation.

In gradient HPLC, serious problems arise in quantification, as the evaporative light scattering detector does not detect lower fatty esters and underestimates the lowest oligomers also in other series of surfactants. This problem can be overcome by using liquid exclusion adsorption chromatography (LEAC), which can be run under isocratic conditions, and thus allows the use of refractive index (RI) detection.

Basically, amphiphilic polymers can be characterized using different chromatographic techniques, which separate according to different criteria.

In the case of amphiphilic materials (such as surfactants like FAE, FAMEE etc.) the following options exist:

The overall molar mass distribution (MMD) can be determined by Size Exclusion Chromatography (SEC), the resolution of which is, however, limited.

The individual homologous series can be separated according to their hydrophobic groups by Liquid Chromatography at the Critical Adsorption Point (CAP) for the (more polar) polyoxyethylene block on a reversed phase column (Liquid Chromatography under Critical Conditions, LCCC).

A separation according to the polar EO chain can be achieved by Liquid Adsorption Chromatography (LAC) on a normal phase column or by Liquid Exclusion-Adsorption Chromatography (LEAC) on a reversed phase column. In the range of lower degrees of ethoxylation LEAC is superior to LAC, as it can be run in isocratic mode (which allows an accurate quantitation), while LAC typically requires gradient elution and thus the use of the evaporative light scattering detector (ELSD), with all the quantitative problems resulting therefrom.

Diesters of PEG can be separated according to the length of the EO block at the CAP for PEG or even better under LEAC conditions (with the hydrophobic groups in adsorption and PEG in exclusion regime).

Obviously, a full characterization of complex samples will often require a combination of at least two different separation modes, such as LCCC-SEC, LCCC-LAC, or LCCC-LEAC.

As has been shown, a full characterization of such products with a baseline separation of all oligomers can be achieved for monofunctionals or two-blocks by 2-dimensional liquid chromatography, and in many cases also for difunctionals or three-blocks.

**L-8**

Miklós S.Z. Kellermayer, Attila Nagy, László Grama,
András Málnási-Csizmadia

# SINGLE-MOLECULE METHODS

*Dept. Biophysics, Univ. Pécs, Faculty of Medicine, Szigeti út 12.,Pécs H-7624 Hungary*
*Dept. Biochemistry, Eötvös Univ., Pázmány Péter sétány 1/c., Budapest H-1117 Hungary*

Single-molecule methods are a unique set of techniques that enable the visualization and manipulation of individual molecules under functionally relevant conditions. Studying single molecules has several advantages over ensemble methods: a) spatial averaging is avoided, which enables the exploration of processes that proceed via parallel pathways, such as protein folding; b) temporal averaging is avoided, which enables the exploration of stochastic processes, such as the blinking of fluorophores; c) anisotropic forces can be applied, which enables the exploration of the elastic properties, mechanically-driven transitions and motor functions of biomolecules. Here we demonstrate various single-molecule techniques through studying the giant muscle protein titin.

Titin is a filamentous protein that spans half of the striated muscle sarcomere, generates passive muscle force upon stretch, and may serve as a template for sarcomere assembly. The molecule is a chain of ~300 globular (Ig or FN) domains and unique sequences, most notable of which is the proline-, glutamate-, valine- and lysine-rich PEVK domain. Fluorescently labeled titin molecules equilibrated onto a glass surface appeared as bright particles with a size of the diffraction limit. From the kinetics of titin's surface adsorption the molecule's translational diffusion constant was estimated. By following changes in fluorescence intensity as a function of time, the kinetics of chemical denaturation were studied at the single-molecule level. Fluorescently labeled strands of titin stretched with meniscus force or by nanomanipulation appeared as strings of bright beads that correspond to compact structures interspersed with faint regions that correspond to loose structural elements. Measurements, with laser tweezers or atomic force microscopy (AFM), of the force required to stretch a single molecule revealed that titin behaves as a highly non-linear entropic spring in which domain unfolding occurs at high (above 30 pN) and refolding at low (~2.5 pN) forces. Comparison of experimental data with predictions of the wormlike chain (WLC) theory revealed a persistence length of ~15 Å for the single unfolded molecule. The behavior of the partially unfolded titin molecule can be well simulated as serially linked WLC's with distinct elastic properties. During repeated stretch-release cycles titin became mechanically worn-out in a process we call "molecular fatigue." Since titin's molecular fatigue occurs in a physiologically relevant force range, the process may play an important role in dynamically adjusting striated muscle's response to the recent history of mechanical perturbations. To explore the mechanical properties of structurally distinct regions in titin, we expressed a recombinant eight-domain segment (octamer) from titin's tandem-Ig region, and three (N-, C-terminal, and middle) ~700-residue-long segments of the PEVK domain. In case of the octamer the mean domain unfolding force, at a stretch rate of 1 μm/s using single-molecule AFM, was 108 pN (±50 SD). Considering that larger unfolding forces have been measured for other regions of the molecule, the octamer may be a mechanically less stable part of titin. The mechanical response of the PEVK segments could be well fitted with the WLC model. The persistence lengths ranged between 0.09 and 1.74 nm. At 0.8 μm/s stretch rate no force hysteresis was observed, suggesting that the PEVK segment is an ideal spring. In sum, single-molecule techniques provide unique insights into the elasticity and structural transitions of biomolecules.

**L-9**

Wolfgang Kern and Claudia Preininger

# PHOTOREACTIVE POLYMER SURFACES FOR IMMOBILIZATION OF BIOMOLECULES

*Graz University of Technology, Institute for Chemistry and Technology of Organic Materials, Stemayrgasse 16.,A-8010 Graz*
*Austrian Research Centers GmbH-ARC, A-24444 Seibersdorf*

The immobilization of biomolecules on solid supports is of importance in the fields of genomics, biomedical technology and surface modification. Polymers capable of binding biomolecules are especially attractive, since polymers can be processed by moulding and thin-film casting techniques. Additional benefits are low fabrication costs and desirable mechanical properties. Depending on the polymer chosen, both rigid and elastic materials are accessible.

We developed copolymers of styrene and 4-vinylbenyl thiocyanate (PST-co-VBT) for the purpose of binding functional molecules onto their surface. After UV irradiation ($\lambda$ = 254 nm), the photoisomerization of the thiocyanate (SCN) groups gives the corresponding isothiocyanates (NCS) in high yield. NCS units are highly reactive towards amines and amino-functionalized molecules and derivatives of thiourea are obtained. The photoreactive polymer PST-co-VBT is unique as the units capable of binding amino-functionalized molecules are generated by UV radiation. Therefore, reactive patterns at the surface are obtainable by lithographic techniques.

We describe the photoinduced reactions in PST-co-VBT and the analytical techniques to follow the SCN-NCS transformation. The reaction is accompanied by significant changes in the optical properties of the polymer, which can be assessed by techniques such as ellipsometry and diffraction of laser light. An interesting surface topography can be obtained by interference patterning as was proved with atomic force microscopy (AFM).

The binding of amino-terminated DNA sequences onto activated films of PST-co-VBT has been studied using fluorescence labelled compounds. The binding capacibility, the generation of arrays and first results of hybridization experiments are discussed with regard to practical applications in the field of DNA chip technology.

**L-10**

Nelli Farkas, Dénes Lőrinczy, Timea Dergez, Franciska Könczöl, Ferenc Kilár, Joseph Belágyi

# EFFECT OF POLYCYCLIC AROMATIC HYDROCARBONS ON ERYTHROCYTE MEMBRANES BY DSC AND EPR

*Institute of Bioanalysis, Institute of Biophysics, Institute of Forencis Medicine, Faculty of Medicine, University of Pécs, Pécs, Hungary*

DSC and EPR experiments were performed on human erythrocyte membranes and DPPC model systems in order to study the effect the polycyclic aromatic hydrocarbons on lipid structure and dynamics. Eight different compounds among others naphthalene and pyrene were compared, which occur in significant concentrations in dust collected from the air in large cities.

Experiments using spin label technique showed that the compounds induced mobility changes in the lipid region in the environment of the fatty acid probe molecules incorporated into the membranes. The effects depended on the structure and concentration of the different compounds. Similarly to EPR observations, DSC measurements reported decrease of transition temperature in comparison to control DPPC vesicles. These results suggest that polycyclic aromatic hydrocarbons were able to modify the internal dynamics of erythrocyte membranes which might lead to damage of the biological functions.

**L-11**

Magyarlaki, T., Rékási, Zs. and Csutora, P., Kellermayer, M.

# ABBOTT CELL-DYN HEMOCYTOMETRY, FLOW CYTOMETRY AND MULTIDRUG RESISTANCE IN B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

*Department of Clinical Biochemistry, University of Pécs, Medical Faculty*
*Ifjuság u. 13. 7624-Pécs, Hungary*
*Phone/FAX: +36-72-536120 / +36-72-536121*
*e-mail: miklos.kellermayer@aok.pte.hu*

The aim of the study was to test the diagnostic potential of morphology (smears and CD4000 cytometry) in the blood samples of 47 B-lymphoproliferative syndrome (LPS) patients (including 41 B-CLL) and 42 controls diagnosed on the basis of their flow cytometric (FACS) features and multidrug resistance (MDR) genetics.

Results: 1./ Nineteen postfollicular and 8 “atypical” B-CLL (27/42) could be diagnosed on simple morphological grounds.

2./ Eleven “atypical” B-CLL and 9 B-LPS (2 B-CLL suspect, 2 mantle zone-, 2 hairy cell- and 2 follicular lymphomas) and 42 controls (14 lymphocytosis, 18 lymphoma remission and 10 lymphadentitis) were diagnosed by FACS phenotype (20/47 and 42/42). In this cases no distinctive morphological features (high absolute lymphocyte count, monomorphic- or “immature” cell morphology, Gumprecht cells, fragile white blood cells, non-viable cells, RBC and/or PLT abnormalities) were present to help the diagnosis.

3./ Additionally, higher number of MDR positive cases were present in the B-LPS group (29/47) - especially in “atipical” B-CLL forms - (15/19) compared to controls (4/42). There were no specific morphological or phenotype features of the chemoresistant cases.

Conclusion: Although the peripheral lymphocyte morphology can be diagnostic in many cases of B-CLL, the flow cytometry with phenotype and MDR tests are obligatory in all cases of B-LPS patients. FACS measurements can give necessary prognostic and differential diagnostic information unpredictable from the morphology alone.

**L-12**

Boldizsár, F., Czömpöly, T., Pálinkás, L., Bartis, D., Németh, P. and Berki, T.

# MULTIPARAMETRIC ANALYSIS OF GLUCOCORTICOID RECEPTOR EXPRESSION IN THYMOCYTE SUBPOPULATIONS

*University of Pécs, Faculty of Medicine, Department of Immunology and Biotechnology, Pécs, Hungary*

Glucocorticosteroids (GCs) produced by the stromal cells of the thymus influence the maturation and selection steps of developing thymocytes by modifying the T cell receptor (TcR) derived signals. There is not yet clear weather the GC action in this process is mediated through the classical glucocorticoid receptor (GCR) induced genomic or the recently described non-genomic (direct membrane or membrane receptor mediated) pathways. We have shown previously that double positive (DP) thymocytes were the most sensitive to Dexamethasone (DX) induced apoptosis in vivo although they express the lowest GCR protein level. The aim of this study was to detect and compare the GCR expression of thymocyte subgroups both at the protein and mRNA levels and to investigate the effect of DX (synthetic GC) treatment on GCR mRNA and protein level.

Therefore 4 week old Balb/c mice were treated with high or low doses of DX ip., and after 24 hours thymi were isolated and homogenized. The thymocytes were labeled with monoclonal anti-CD4PE (FL2) and anti-CD8CyC (FL3) antibodies to distinguish the four characteristic thymocyte subpopulations: the most immature CD4-CD8- double negative (DN), the CD4+CD8+ double positive (DP) and the mature CD4+ or CD8+ single positive (SP) cell groups. Thymocyte subgroups were separated (sorted) according to their FL2-FL3 fluorescence using the BD FACSVantage Cell Sorter instrument. From the pure (98-99%) thymocyte subpopulations (DN, DP, CD4 or CD8 SP) we isolated RNA, then reverse transcription was performed followed by real-time PCR analysis of the mRNA levels of the cells. We also examined the time course of GCR mRNA expression after 30min, 1, 2, 4, 8, 12, 16, 20, 24h of a single high dose DX treatment. Cytoplasmic GCR levels were determined after intracellular anti-GCR-FITC labeling in combination with anti-CD4-PE and anti-CD8-CyC monoclonal antibodies, followed by triple colour fluorescence analysis.

DN thymocytes expressed the highest level of GCR mRNA followed by CD8 SP, CD4 SP and DP thymocytes. DP cells had the lowest cytoplasmic GCR level, together with low GCR mRNA expression. After 24 hours of high or low dose DX treatment DN, CD4 SP and CD8 SP cells showed significant decrease of GCR mRNA expression (75%, 66% and 80% decrease respectively). DP thymocytes, on the other hand, showed no significant alteration in GCR mRNA expression after either dose DX treatment. Interestingly, there was a transient, significant increase in the GCR mRNA level of thymocytes after a single high dose DX treatment at 8h, followed by a significant decrease at 24h.

The different GCR mRNA expression of thymocyte subgroups was in line with our previous flow cytometric findings on the cytoplasmic GCR protein levels. The down regulation of GCR expression seems to be a common mechanism in DN, CD4 SP and CD8 SP cells, but not in DP thymocytes. DP cells are unable to down regulate GCR levels in the presence of GC hormone, thus they are more sensitive to GC induced apoptosis. The low GCR expression of DP thymocytes together with the high sensitivity also raises the possibility of non genomic GC action.

**L-13**

Ernst Haslinger

# PHARMACEUTICAL APPLICATIONS OF NMR-SPECTROSCOPY

*Institute of Pharmaceutical Chemistry*
*University of Graz (Austria)*

The lecture is divided in three parts. Each showing a different aspect of the application of NMR spectroscopy in the pharmaceutical sciences. NMR can be used for structure determination of bioactive natural products, for the investigation of the functional structural changes of proteins and for the determination of biochemical processes like gluconeogenesis in man.

In the first part structure determination of natural products will be discussed. Emphasis will be laid on the structure determination of molecules with complex carbohydrate structures. It will be shown, how NMR can provide structural information about oligosaccharide structures by combining COSY- and TOCSY-experiments. Selective excitation can be used to simplify the spectra considerably.

The second part is concerned with the investigation of the structural behavior of metallothioneins. Metallothioneins are small, cysteine rich metal binding proteins, which release metal ions by interaction with nitric oxide. To study the structural changes a mouse [$Cd_7$]-metallothioneine sample was titrated with an NO donor and monitored by NMR spectroscopy. It will be shown how $^{113}$Cd-$^{1}$H-HMQC can be used to characterize the structural changes of potential physiological significance.

The third part is devoted to the application of $^{2}$H-NMR spectroscopy and its use in the measurement of fractional whole-body gluconeogenesis (GNG) in humans. In type 2 diabetic patients, GNG primarily accounts for increased endogenous glucose production and fasting plasma glucose concentrations. Determination of the fraction GNG contributes to the whole blood glucose will give information about the importance of these metabolic pathways. Previous methods for measuring GNG in man used the determination of $^{2}$H at carbons 5 and 2 of blood glucose after ingestion of $^{2}H_2O$, using gas-chromatography and mass spectrometry. It will be shown how $^{2}$H NMR spectroscopy can be used to determine the GNG in man. The results are compared with results of in vivo $^{13}$C-NMR spectroscopy and the above mentioned GC-MS-method.

**L-14**

László Kollár

# $^{31}$P NMR INVESTIGATION OF PLATINUM(II) COMPLEXES CONTAINING MULTIDENTATE LIGANDS

*Department of Inorganic Chemistry, University of Pécs, H-7624 Pécs, Ifjúság u. 6., Hungary*
*e-mail: kollar@ttk.pte.hu*

The importance of homogeneous catalytic reactions, among them carbonylation reactions has initiated many studies describing mainly the range of applicability as well as mechanistic considerations [1]. Large efforts have been made to exploit hydroformylation reaction in the synthesis of chiral building blocks and biologically important derivatives [2].

Cobalt and rhodium complexes are still dominating as hydroformylation catalysts, but the use of platinum-phosphine-tin(II)chloride systems are promising. Hundreds of ligands with different steric and electronic properties, shapes and functionalities have already been tested in catalytic reactions [3], including enantioselective hydroformylation [4].

The most frequently used ligands in transition metal complexes possess two chemically equivalent phosphorus donor atoms due to their c2 symmetry (‚homobidentate ligands') and the minority of the bidentate ligands are mostly P,N or P,O donors (‚heterobidentate ligands'). The common feature of these ligands is their coordination in a *cis*-manner in complexes with squareplanar geometry.

The aim of this paper is to present some recent results on the structural characterisation of the platinum(II) complexes with various multidentate ligands in solution.

$^{31}$P NMR characterization of novel neutral and ionic platinum(II) complexes including PPP (bis(2-diphenylphosphinoethyl)-phenylphosphine), $PP_3$ (tris(2-diphenylphosphino-ethyl)-phosphine), PPN (1,1'-bis(diphenylphosphino)-α-N,N-dimethylamino-[2,3]-tetramethylene-ferrocene) ligands will be shown. The multidentate ligands might act as bridging ligands in tetragonal multinuclear complexes or might force the Pt(II) complexes in unexpected trigonal bipyramidal geometry.

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**L-15**

Zoltán Berente, Erzsébet Ősz, Balázs Sümegi

# ENERGY METABOLISM STUDIES IN LANGENDORFF PERFUSED RAT HEART USING IN SITU $^{31}$P NMR

*Department of Biochemistry and Medical Chemistry, University of Pécs*

Nuclear magnetic resonance (NMR) is a powerful tool for *in situ* and *in vivo* metabolic studies since it employs radiofrequency radiation which is a non-ionizing radiation and it is not considerably attenuated in tissues. Therefore, information can be obtained from the sample at macroscopic, microscopic and molecular level as well in a *non-invasive* and *non-destructive* manner.

Studies reported in the presentation pursued the beneficial effects of some poly ADP-ribose polymerase (PARP) inhibitors on the viability of Langendorff perfused rat hearts after ischaemia and reperfusion. Phosphocreatine, ATP and inorganic phosphate levels were monitored *in situ*, and their recovery after reperfusion was quantified and compared to the normoxic value.

The presentation intends to provide some insight into the molecular mechanism of the phenomenon and the trade-off between perision and biological relevance will be discussed as well by comparing *in situ* results to those obtained *ex vivo* by earlier methods.

**L-16**

Sándor Kunsági-Máté, Géza Nagy, László Kollár

# CONFORMATIONAL AND SOLVENT EFFECT ON THE COMPLEXATION ABILITY OF SOME FUNCTIONALIZED CALIXARENS SHOWING SENSOR ACTIVITY TOWARDS NEUTRAL GUESTS

*Department of General and Physical Chemistry, University of Pécs, Hungary*
*Department of Inorganic Chemistry, University of Pécs, Hungary*
*MTA-PTE Research Group for Chemical Sensors, Pécs, Hungary*
*Mail: 7624 Pécs, Ifjúság u. 6, Hungary; E-mail: kunsagi@ttk.pte.hu*

The recognition of neutral organic molecules by synthetic receptors is a topic of current interest in supramolecular and also in analytical chemistry. Calixarene derivatives are well- known host molecules with photoluminescent (PL) behaviour at near ultraviolet range. Knowing that fluorescence generation is a very sensitive signal-forming technique, studies have been carried out with different calixarenes and organic guest molecules to collect data about the influence of the supramolecular interaction on the PL signal.

The 'host' properties of distally substituted calix[4]arene and 4-tert-butyl-calix[4]arene with different neutral pesticide related trifluoromethyl-benzene (benzotrifluoride) derivatives as 'guests' have been investigated earlier in chloroform, dimethylformamide and in different alcoholic solvents.1-3 The complex stability constants (Ks) and the thermodynamic parameters of the complexation were determined by PL measurements using Job's method and van't Hoff theory, respectively.3 The host-guest interaction and complex formation were checked by quantum-chemical method. The calculations indicated that the host cavity changes its conformation interacting with the neutral guest molecules. The 'pinched cone' conformer of 1,3-substituted calix[4]arenes seems to form a complex with electron-deficient neutral aromatics by predominant π-π type interaction. As it was found previously, the nature of interaction is highly affected by the solvent permittivity.2

As a part of our continuing interest, the interaction of a water-soluble C-methyl-calix[4]resorcinarene (1) with some simple aromatic compounds has been examined. Interestingly, interaction in water was not found. This phenomenon can be understood by considering the high permittivity of water and by our earlier findings showing that the higher the permittivity the lower the complex stability.3 Since cation-π linked sandwich complexes of iron are listed 4 among reversible electrochemical mediators, we examined the possible interaction of 1 with aromatic guests under the presence of iron ions. The coordination of Fe(II) and Fe(III) ions towards 1 has been investigated. The related results suggest that Fe(II) ions form complexes with 1 through cation- π interactions, while the complexation of Fe(III) ions is mainly based on the chelate formation with the carboxylate moieties of the resorcinarene molecule. 5

The results show that Fe(II) ions support the interaction of 1 with some neutral aromatic compounds. Related quantum-chemical investigations suggest that the complexation occurs by the formation of a sandwich-like complex between a resorcinarene ring and the ring of the aromatic guest by the assistance of Fe(II) ions.

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**L-17**

R.Ohmacht, Z. Szabo, L. Mark, W. Stöggl, Ch. Huck, G.K.Bonn

# EFFECT OF THE PORE STRUCTURE ON THE BEHAVIOUR OF LONG CHAIN ($C_{18}$, $C_{30}$) REVERSED PHASE HPLC PACKINGS

*Institute of Biochemistry and Medical Chemistry, University Pecs, H-7624 Pecs, Hungary*
*Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, A-6020 Innsbruck, Austria*

Reversed phase chromatography is by far the most popular mode of high-performance liquid chromatography (HPLC). The most widely used packings are those made of porous silica chemically surface modified with hydrocarbon chains. Although reversed phase (RP) stationary phases become commercially available in the early seventies there are still work in progresses to improve the quality and reproducibility of nonpolar, silica based packing materials. It has been demonstrated that pore size and pore structure of starting silica plays an important role in the behaviours of these stationary phases. Therefore experiments were carried out on silicas with different pore diameter in the range 9.3-25.5 nm. The starting silicas were prepared by hydrothermal procedure. The reaction yields silicas with predictable pore size and surface area. The porosity data of the phases shows that the silylation with the octadecyl/triacontyl-silane considerably changes the pore structure of the silica. All the pore size, pore volume and surface area decrease. According to the test elaborated by Sander & Wise our phases belong to the intermediate, densely loaded, monomeric type of phases. Endcapping does not change remarkably the average pore size of the samples, in general pore volumes and surface areas show a small further decrease. Beside "classical" surface coverage (calculated from the carbon content and surface area of the phases) we introduce a new parameter: **volumetric surface coverage [**$mm^3 \times m^{-2}$**]**. This parameter shows the occupied volume of the pores of silica gel by the hydrocarbon chains. The phenol-pyridine test demonstrates that even by high loaded phases good separation of basic compounds can be achieved after exhaustive endcapping procedure only. The separation capabilities of the phases will be demonstrated by different demanding separations e.g. flavonoids, tocopherol isomers (α,β,γ,δ), fullerenes, anions.

**L-18**

M. Gfrerer, D.Bandoniene, E.Lankmayr

# COMPARATIVE STUDY FOR THE DETERMINATION OF POLAR PESTICIDES

*Institute for Analytical Chemistry, Micro- and Radiochemistry, Graz University of Technology , Technikerstr. 4/P, 8010 Graz, Austria*

Due to their large scale application the analysis of polar acid compounds has become important to environmental chemists in the last decade. Among these compounds the chlorophenoxy acid herbicides are widely used in agriculture, industrial weed-control and forestry. Owing to their physical properties they are frequently detected in surface and groundwater near agricultural fields or golf courses. Although the chlorophenoxy acids (CPAs) are directly amenable to HPLC or capillary zone electrophoresis, the demand for low limits of detection in environmental analysis logically leads to GC techniques. Unfortunately the CPAs are thermally unstable during GC analysis and require, therefore, a preliminary derivatization step. The most representative derivatization processes are esterification, alkylation, acylation or silylation. Esterification involves nucleophilic substitution generally catalyzed by a strong acid ($H_2SO_4$) or by a Lewis acid ($BF_3$). Diazomethane alkylation, the standard method by US EPA, also consists of nucleophilic substituion but a bimolecular mechanism is involved, without formation of a carbocation. A rather new aproach is the injection-port derivatization procedure with an ion-pair reagent. In the present study different derivatization procedures, which form chlorophenoxy acid methyl esters, are compared with regard to reaction yield, limit of detection and sensitivity. Beside the classic procedures, which require reaction times up to two hours, microwave-enhanced esterification methods are presented and included in the comparison.

**L-19**

Karin Eggenreich, Reinhold Wintersteiger

# DEVELOPMENT OF COSMETICLY USED AMINOCRESOLS AND METABOLITES IN BIOLOGICAL MATERIALS BY HPLC COMBINED WITH UV FLUORESCENCE AND MS DETECTION

*Institute of Pharmaceutical Chemistry and Pharmaceutical Technology, University of Graz*

For the determination of 4-amino-m-cresol (4-AC) and 5-amino-o-cresol (5-AC), two oxidative hair dyes, highly sensitive analysis methods had to be developed. In order to be able to quantificate these compounds in plasma in the picomole range two different fluorogenic agents, OPA and fluram, were tested due to their ability for fluorescence derivatization. Numerous optimization steps regarding reaction time, reaction conditions, detection wavelengths and molar excess of reagent were carried out. Using OPA a reaction time of 20 minutes at room temperature in a borate buffer pH 10.2 proved to be optimal. The substances under investigation showed different LODs of 10 and 500 picogram, resp., for 4-AC and 5-AC. Using fluram a reaction time of 5 minutes at room temperature in a borate buffer pH 8.0 proved to be optimal. LODs were 10 picogram for 4-AC and 1 nanogram for 5-AC. Coefficients of variation were below 5%. To determine 4-AC and 5-AC in plasma solid phase extraction was performed on C18 PolarPlus cartridges resulting in recovery rates higher than 70% applying HPLC and UV detection.

Metabolism studies were carried out in two different biological materials producing different metabolites. Therefore, S9 (rat liver homogenisate) and HaCaT (human keratinocytes) were analyzed by HPLC/MS under further optimized chromatographic conditions. Investigations with S9 were possible with isocratic technique whereas for HaCaT a gradient system had to be developed. For HaCaT the N-acetylated derivative of 4-AC and 5-AC was identified as main metabolite and for S9 16 different metabolites of the aminocresols investigated were found. The ionisation technique used for MS analysis was APCI.

**L-20**

Michael Murkovich

# ACRLYAMIDE IN AUSTRIAN FOODS

*Graz University of Technology, Department of Food Chemistry and Technology*
*Petersgasse 12/2, 8010 Graz, Austria*
*Tel: +43 316 8736495, Fax: +43 316 8736971, Email: michael.murkovic@tugraz.at*

Acrylamide from foods was identified as a possible cancer risk in the beginning of 2002. The Swedish Food Authority was the first to report high concentrations of acrylamide in foods. With this background a study on Austrian foods was carried out. The analysis was carried out using liquid chromatography for separation of the acrylamide on a reversed phase column and mass selective detection with electrospray for ionisation.

The concentrations found in Austrian foods ranged from 0 to 2410 ng/g with the highest concentrations being in potato chips. Cookies contain about half of the acrylamide compared to potato chips and breakfast cereals, bread and similar products and popcorn having even lower concentrations. Pizza and Wienerschnitzel – two typical Austrian foods – have very low amounts of acrylamide with most of the samples lying below the limit of detection. Coffee which contributes significantly to the exposure to acrylamide contains ca. 200 ng/g.

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**L-21**

Anikó Takátsy, Ferenc Kilár

# CHEMICAL STRUCTURE AND MOLECULAR RECOGNITION: CAPILLARY ELECTROPHORESIS AND MODEL CALCULATION STUDIES

*Institution of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12., H-7624 Pécs, Hungary*

Application of proteins as stereoselective selectors provides separation of optical isomers and information about the molecular surface properties. We have studied the interactions of human serum transferrin with tryptophan-methyl, -ethyl, -butyl esters by capillary electrophoresis and model calculations. The iron-binding site of the protein is responsible for the chiral recognition, but the experimental results, as well as, the model calculations reflected a difference in the separation of tryptophan derivatives having different alkyl chains. The interaction was followed by the electrophoretic migration of the ligand molecules through a pseudostationary transferrin zone and changes in the electrophoretic mobility were observed. The enantiomeric resolution was investigated changing the experimental parameters, e.g., migration time, length of the transferrin zone, concentration of the protein, etc. We obtained that the length of the alkyl chain might influence the interaction of the molecules with the transferrin surface.