ABSTRACTS

POSTERS

P-1 - P-82

P-1

I. Bacskay, A. Takátsy, A. Elfwing, A. Ballagi, F. Kilár, S. Hjertén

POLYACRYLAMIDE GELS AS "ARTIFICIAL ANTIBODIES" AGAINST PROTEINS AND BACTERIA

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Synthesis of "artificial antibodies". Hemoglobin (Hb) is added to the monomer solution. Following the polymerization the gel is granulated and the hemoglobin is removed. The cavity formed has both a shape and binding sites complementary to those of the hemoglobin molecule and, therefore, recognizes this protein, but not other proteins.

Detection of a selectively adsorbed protein by staining. Gel granules were synthesized in the absense of Hb (blank), (2) in the presense of Hb, followed by removal of the protein by trypsin; (3) as in (2) followed by application of Hb and washing with buffer; (4) as in (3), but albumin and phycoerythrin were added instead of Hb. In these experiments only gel (3) could be stained with Comassie Brillant Blue.

Conclusions: Gels can be synthesized to be selective for a given protein (in this case hemogobin); staining is a simple detection technique.

Selectivity of artificial antibodies, as studied by capillary electrophoresis. Selectively adsorbed bacteria make the neutral polyacrylamide gel granules negatively charged. The eletrophoretic migration velocity is, therefore, a measure of the amount of bacteria adsorbed (Fig. 1). This experiment indicates that polyacryamide gels can be made selective also for particles, such as E. coli bacteria.

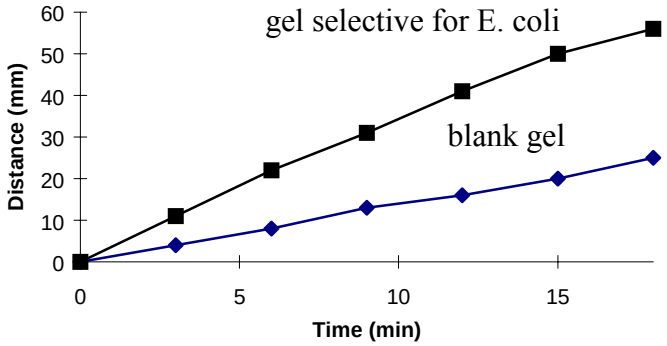

Figure 1.

P-2

D. Bandoniene, M. Gfrerer, E. Lankmayr

COMPARATIVE STUDY OF TURBULENT SOLID-LIQUID EXTRACTION METHODS FOR THE DETERMINATION OF ORGANICHLORINE PESTICIDES

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The aim of any extraction method in analytical chemistry is to effectively separate the analytes from the matrix with minimal solvent and time required. In recent years, for the extraction of organic trace compounds from solid matrices, the classic Soxhlet extraction has been replaced by faster, less solvent consuming and often automated techniques [1, 2].

In this study, a comparison of the classic Soxhlet extraction and some new turbulent solid-liquid extraction techniques, such as fluidized-bed extraction (FBE), modified dive-in fluidized-bed extraction (dive-in FBE), modified dive-in Soxhlet extraction (dive-in Soxhlet) and thimble extraction for the determination of organochlorine pesticides was carried out. The turbulent extraction methods were performed by using the fexIKA Vario control series extractor and modification of the extraction system respectively. In addition, FBE and dive-in FBE were operated under just the same optimum conditions, which had been established for the FBE system, the experimental parameters being the number of extraction cycles, the composition of the extraction solvent and the holding time of the extraction phase after reaching the heating temperature. For the determination of the analytes a selective clean-up of the extracts followed by a gas chromatography method with mass spectrometric detection was used. All the advanced extraction methods with reduced time and low solvent consumption exhibited higher extraction efficiency than the standard procedure, Soxhlet extraction.

[1] Martens D, Gfrerer M, Wenzl T, Zhang, Gawlik BM, Schramm KW, et al. Comparison of different extraction techniques for the determination of polychlorinated organic compounds in sediment. Anal Bioanal Chem 2002;372:562-568.

[2] Gfrerer M, Stadlober M, Gawlik BM, Wenzl T, Lankmayr E. Enhanced extraction of polychlorinated organic compounds from soil samples by fluidized-bed extraction (FBE). Chromatographia 2001;53:442-446.

P-3

M. Bernardo, G. Kollenz and K. Francesconi

^{1}H NMR SPECTROSCOPY: A GUIDE FOR THE DESIGN OF A ONE-POT SYNTHESIS OF ^{14}C-ARSENOBETAINE BROMIDE

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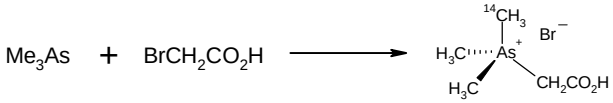
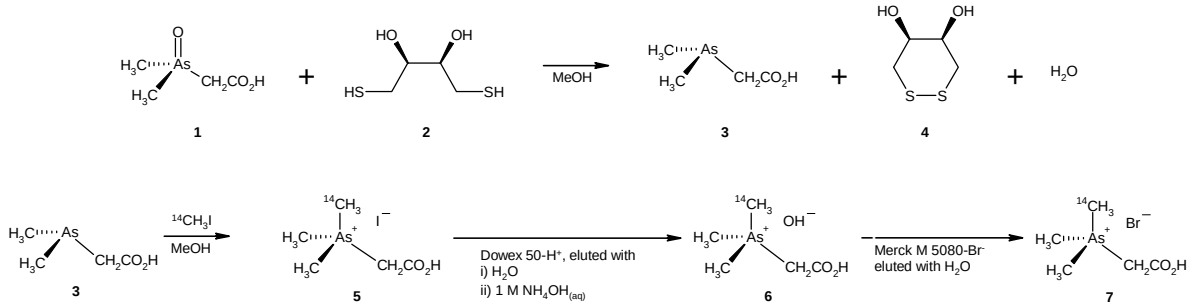
Arsenobetaine is one of the most common arsenicals found in marine organisms [1], although its origin and biological role are as yet unclear. Studies investigating these aspects used arsenic speciation analysis to determine distribution and biotransformation of arsenic compounds, but few studies employed radiolabelled compounds. [2] The use of a radiolabel can provide additional information on uptake and whole body distribution of a compound, such as arsenobetaine. The previously published synthesis for arsenobetaine bromide involves the quaternization of trimethylarsine with 2-bromoacetic acid: [3]



This synthesis is not easily adapted to the synthesis of 14C-arsenobetaine bromide, as the precursor 14C-trimethylarsine is an air-sensitive, low-boiling liquid. It may be difficult to handle, and loss of precious radiolabelled material is imminent. Thus, arsenobetaine bromide was prepared by an alternate route, wherein the 14C-radiolabel was introduced in the final reaction step:



Preliminary experiments, in which dimethylarsinoylacetic acid (1), dithioerythritol (2) and (normal) MeI were allowed to react for times ranging from 5 min to 1 hour, gave poor yields. This indicated that the reduction of 1 or quaternization of the arsine 3 under these conditions did not proceed as quickly as previous experience had suggested. [4] ^{1}H NMR studies were therefore undertaken in order to determine suitable reaction times for both the reduction and quaternization reactions. The reduction of 10 mg 1 by 1.1 eq of 2 in 750 µL D2O was found to be essentially complete within 15 minutes. By contrast, the quaternization of the arsine 3 occurred to the extent of 20% in the presence of 1 equivalent of MeI, and 83% with 10 equivalents of MeI, after 30 min. In our protocol, 1 mmol of 1 was reacted with 1.5 mmol 2 for 1 h, then with 1.1 mmol MeI for 24 h, followed by work-up using cation- and anion-exchange columns. The final product **7** was isolated in 22% yield.

[1] Francesconi, K. A.; Edmonds, J. S. *Adv. Inorg. Chem.* **1997** *44*, 147–189.

[2] See for example: Challlenger, F. et.al., *J. Chem. Soc.* **1954**, 1760; Cullen, W.R. et al., *J. Organomet. Chem.* **1977**, *139*, 61; *Can. J. Chem.* **1979**, *25(10)*, 1201; Vahter, M.; Norin, H. *Environ. Res.* **1980**, *21(2)*, 446.

[3] Minhas, R. et al. *Appl. Organomet. Chem.* **1998**, *12(8/9)*, 635.

[4] Francesconi, K. A. et al. *Appl. Organomet. Chem.* **1994**, *8(6)*, 517.

P-4

L. Binder, G. Rathwallner, B. Mollay, G. E. Nauer, D. Lang

THE USE OF RDE-EXPERIMENTS AS A TOOL TO STUDY THE ELECTROCHEMICAL DISSOLUTION OF MOLYBDENUM IN ALKALINE ELECTROLYTES

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The objective of this project is to find a model for the electrochemical dissolution of molybdenum in alkali-hydroxide-solutions which allows the prediction of the current-potential behavior for different process parameters. The results should raise a better understanding of the dissolution process, e-g. speed limiting steps, being the base for optimization or redesign of electrochemical treatment.

Experimental work: Current-potential curves are measured using a Molybdenum rotating disk electrode in potassium hydroxide solutions of different concentration and with addition of different amounts of salts (e.g. K_2MoO_4 and Na_2CO_3). Experiments are carried out in air-saturated solutions. By ernployment of different electrochemical methods (e.g. cyclic linear sweep voltammetry and steady state methods) basic information about the etectrode processes was available.

Simulation: The current-potential curves are fitted for different rnodels of electrochemical oxidation and solvation with the PIRoDE-software. The resulting fits are investigated concerning their suitability for different rotation speeds, changed bulk concentrations and varying scan rates.

PIRoDE allows to calculate the distribution of electrolyte ions after input of the relevant parameters. These calculations are made for direct and alternating current as well as for different current pulses, basing on the approved models of dissolution. The models are either verified or redesigned and tested again.

Results: Finally, different models of electrochemical dissolution of molybdenum are presented and discussed, based on the rotating disk results in combination with the modelling process.

P-5

I. Bíró, V. Poór, A. Bufa, Á. Gáti, I. Fenyvesi, S. Juricskay, T. Tényi

URINARY STEROIDS IN YOUNG WOMEN WITH EATING DISORDERS

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Eating disorders are important health concern among adolescents. Young women frequently present with signs and symptoms of anorexia nervosa, bulimia nervosa and bulimarexia, which are characterized by abnormal eating patterns, depressive and anxious symptoms, enhanced aggressiveness and endocrine alterations, which, in general, result from the body's adaptive response to malnutrition. Endocrine alterations include disorders of metabolism, alteration in cortisol, leptin and neurosteroid metabolism, fluid and electrolyte homeostasis, thyroid function, glucose regulation, growth and development and reproductive function with the development of amenorrhoea as well as the risk of osteoporosis.

The aim of the present study is to obtain comprehensive information on steroid metabolism in patients with eating disorders.

Urinary steroid components were measured after enzyme hydrolysis and methoxym-silyl derivatization by capillary gas chromatography. Using three internal standards, programmed temperature from 50 oC to 300 oC and FID detection, on ULTRA-1 capillary column the separation of 28 steroid components is possible. 15 young women with different eating disorders (anorexia nervosa, bulimia nervosa, bulimarexia) (aged 21.5 ± 1.5) and 15 healthy women (aged 23.4 ± 0.4) collected 24-h urine.

A significantly decreased value ($p<0.05$) of dehydroepiandrosterone (DHEA) was found, the level of stress marker allo-tetrahydrocorticosterone (aTHB) was significantly elevated ($p<0.05$), but the free cortisol level was unchanged in patients with eating disorders.
Our results confirm the role of DHEA in eating disorders. The increased level of the stress marker aTHB refers to high stress-sensibility, but he relevance of hormone alteration to the patophysiology of eating disorders remains to be elucidated.

P-6

V. Tomečková, P. Perjesi, J. Guzy, J. Kušnír, Z. Chovanová, Z. Chavková, M. Mareková

COMPARISON OF EFFECT OF SELECTED SYNTHETIC CHALCONS ON MITOCHONDRIAL OUTER MEMBRANE DETERMINED BY FLUORESCENCE SPECTROSCOPY

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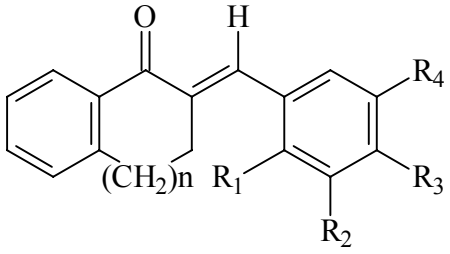
Effect on mitochondrial outer membrane of six selected synthetic cyclic chalcone analogues were investigated by fluorescence spectroscopy. The selected derivatives are representatives of *E*-2-arylmethylene-1-tetralones (n=1) and *E*-2 arylmethylene-1-benzosuberones (n=2).



The main goal of this study was experimental measurement of autofluorescence of the six investigated cyclic chalcones with various aromatic substituents (methyl and methoxy) in the *ortho* and *para* positions. The compounds were dissolved in DMSO, added to a mixture of the respiration medium and succinate, and synchronous and emission fluorescence spectra were recorded. The chalcones were investigated to determine their excitation and emission maxima for measurements of fluorescence polarization, and compare their fluorescence determined by synchronous fluorescence spectra and emission fluorescence spectra. These selected rigid analogues of natural chalcones are conjugated molecules with two aryl rings. Their fluorescense was found to be enhanced by the methoxy (OCH_3), however, reduced by the methyl (CH_3) substituents.

Interaction of subtoxic concentration of the chalcones with the outer mitochondrial membrane was investigated by fluorescence polarization. We monitored changes of fluorescence polarization of the chalcones after their addition to mitochondria over a 30-minute period of time. The chalcones *E*-2-(4′-methylbenzylidene)-1-tetralone, *E*-2-(4′-methoxybenzylidene)-1-tetralone, *E*-2-(2′,4′-dimethoxybenzylidene)-1-tetralone, *E*-2-(4′-methylbenzylidene)-1-benzosuberone, and *E*-2-(2′,4′-dimethoxybenzylidene)-1-benzosuberone displayed an elevated fluorescence polarization. During the 30-minute period, however, we observed depression of the measured values. In case of the chalcone *E*-2-(4′-methoxybenzylidene)-1-benzosuberone, a continuous increase of fluorescence polarization could be observed during 30 the experiments. It was the chalcone *E*-2-(2′,4′-dimethoxybenzylidene)-1-tetralone, which showed the highest changes in fluorescence polarization under the experimental conditions.

The synchronous and emission fluorescence spectra as well as the fluorescence polarization measurement results of the investigated chalcones showed the compounds to be fluorescent. It was found that the ring size as well as the nature and the location of the aromatic substituents have significant effect on their fluorescence and their interactions with mitochondria.

P-7

L. Czimbalek, A. Visegrády, B. Somogyi

THE EFFECT OF PHOSPHOLIPASE C INHIBITION ON REGENERATIVE INTRACELLULAR WAVES

University of Pécs, Faculty of Medicine, Department of Biophysics,
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In electrically non excitable cells, receptor activation often leads to elevated cytosolic free calcium concentration. This increase in calcium level can take the form of complex spatiotemporal patterns, such as propagating wavefronts, that arise through coordinated release of calcium from distinct release sites on the endoplasmic reticulum. The molecular basis of positive feedback effects resulting in the travelling concentration profiles, as e.g. the identity of the diffusing messenger, are not clear yet. One proposed mechanism suggests that the diffusion of locally produced inositol 1,4,5-trisphosphate couples neighboring release sites. Localized and temporally controlled action of the inositol 1,4,5-trisphosphate-producing enzyme phospholipase C is therefore crutial for producing intracellular calcium waves.

Our aim was to characterize the role of phospholipase C in the initiation and propagation of agonist-induced calcium signals in a human epithelial cell line. The effect of the putative phospholipase C inhibitor U73122 was investigated using confocal microscopy in fluo-3-loaded HEp-2 cells. By applying submaximal doses of U73122 we investigated the amplitude, velocity and spatiotemporal profile of ATP-induced calcium signals.

This work was supported by the Hungarian Academy of Sciences.

P-8

L. Czimbalek, B. Bugyi, J. Orbán, A. Lukács, L. Grama, B. Somogyi

FLUORESCENCE ANISOTROPY IMAGING USING A CONFOCAL LASER SCANNING MICROSCOPE

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During fluorescence polarization measurements a fluorophore system is excited by a linearly polarized light, and the polarization state of the emitted fluorescence is detected. These measurements can provide useful information on molecular mobility, size, shape and flexibility of molecules, fluidity of a medium and order parameters (e.g. in a lipid bilayer).

Fluorescence polarization is routinely measured in spectrofluorimeters, where the sample solution is excited by a vertically polarized light, and the horizontally and vertically polarized components of the fluorescence intensity (I_H and I_V) are measured. The polarization state of the fluorescence is characterized by the emission anisotropy, defined as

$$r = \frac{I_V - I_H}{2I_V + I_H}.$$

For accurate measurements a correction factor (G) must be introduced that accounts for the different sensitivities of the detector for different polarization planes. The anisotropy modified accordingly will be

$$r = \frac{I_{VV} - G \cdot I_{VH}}{I_{VV} + 2G \cdot I_{VH}}, \; G = \frac{I_{HV}}{I_{HH}},$$

where the first and second index denotes the direction of the excitation and emission polarization plane respectively.

Our aim was to work out the theoretical background and instrumental development necessary to perform fluorescence anisotropy imaging by using a confocal laser scanning microscope (CLSM). Contrary to bulk measurements in a cuvette, such a measurement would allow us to construct a two- or three-dimensional fluorescence anisotropy distribution of the sample. When examining live specimens (cells) light scattering will also be significantly reduced.

While for the CLSM fluorescence anisotropy is given by the same formula (see above), the correction factor is different. For finding the correct formula for G one must take into account the difference in light paths: fluorescence is detected at 180º in the CLSM (epi-fluorescence), while this angle is 90º in the spectrofluorimeter. Another assumption is that the x and y axes of the microscope stage coincide with the horizontal and vertical polarization directions of the excitation light, and are co-aligned with the horizontal and vertical detector axes. With these conditions the correction factor is

$$G = \sqrt{\frac{I_{VV} I_{HV}}{I_{VH} I_{HH}}}.$$

The validity of the calculations will be tested by comparing the results of bulk measurements obtained by a spectrofluorimeter with those obtained by the modified CLSM system.

P-9

B. Csóka, G. Nagy

METHODS FOR DETERMINATION OF DIFFUSION COEFFICIENTS OF ELECTROCHEMICALLY REVERSIBLE AND IRREVERSIBLE REDOX SPECIES USING SCANNING ELECTROCHEMICAL MICROSCOPY (SECM)

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From scientific research to industrial applications several procedures requires the exact knowledge of diffusion coefficient values. Two methods will be presented here to determine the diffusion coefficient of electroactive species. Both methods are based on precision movement of the microelectrodes in the electrochemical cell using SECM moving capabilities.

Electrochemically reversible species were generated on a macro size platinum electrode by applying a 200 msec impulse. An appropriate potential was set on the detector microelectrode (25 μm diameter) and the current transient caused by the redox species was measured. Doing measurements at known distances between the two electrodes the diffusion coefficient can be calculated by measuring the time from generation impulse to the peak currents at different distances.

Diffusion coefficient of electrochemically irreversible species, like H_2O_2, was also investigated by the mentioned instrumental setup successfully. In this case – instead of generation - a small drop of the measured species was injected into the electrochemical cell.

Both methods were used in aqueous solutions and in agarose gel too. The effect of ion-exchangers for the diffusional speed of the investigated species was also measured.

P-10

J. Deli, P. Oláh, P. Molnár, Gy. Tóth

INVESTIGATION OF THE CAROTENOID COMPOSITION IN PROCESSED PAPRIKA (*CAPSICUM ANNUUM*)

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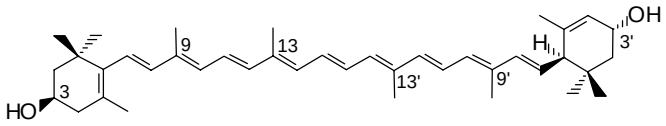
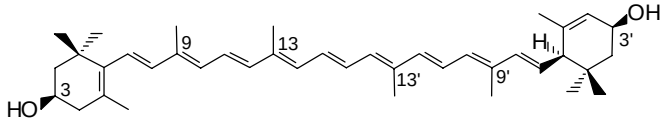
Epidemiological studies have shown inverse correlations between the consumption of vegetables and fruit rich in carotenoids and the incidence of cancer and cardiovascular diseases. Most vegetables are processed rather than eaten raw. Processing includes household cooking and industrial canning, freezing, dehydration, and pickling. These processes involve thermal treatment and exposure to oxidation, which is enhanced by light or the presence of enzymes.

Earlier we have investigated the carotenoid composition of different coloured paprikas (Capsicum annuum). Our investigation showed three characteristic carotenoid compositions. In all unripe (green) fruits of different kinds of paprika, the typical chloroplast pigments, lutein and β-carotene are the main carotenoids. The ripe yellow paprika, whose colour never turns red, contains violaxanthin, antheraxanthin, zeaxanthin, lutein, α- and β-cryptoxanthin, and α- and β-carotene as main carotenoids. In the red paprika, the red colour is due to the carotenoids with κ-end group(s). The main carotenoids are capsanthin, zeaxanthin, cucurbitaxanthin A, β-cryptoxanthin and β-carotene [1].

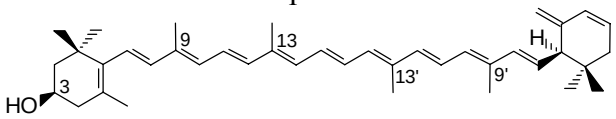
In the present work we report on the carotenoid analyses of cooked, roasted and pickled paprikas (green, yellow and red varieties of Californian paprika (Capsicum annuum var. grossum)). Special attention was payed to the occurrence of minor carotenoids. In the green and yellow coloured paprika, the formation of 3'-epilutein was observed. In addition, in the green paprika the formation of anhydrolutein I was detected.



Lutein



3'-Epilutein



Anhydrolutein I

[1] J. Deli, P., Molnár: *Current Organic Chemistry* 6, 1197-1219 (2002)

This study was supported by the grant OTKA T 037654 and by F Roche Vitamins Ltd (Basel).

P-11

P. Dittrich, F. Legat, F. Tomaselli, A. Maier

MICRODIALYSIS STUDY OF THE DISTRIBUTION OF ANTIBIOTICS INTO INFLAMED TISSUE IN SEVERE INFECTIONS

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Increasing resistance to multiple antibiotics is a world wide problem. Among the contributing factors to the development of bacterial resistance are inadequate concentrations of the antibiotics. A key prerequisite for the optimisation of adequate dosage regimens are pharmacokinetic parameters, usually determined in the central compartment. However many clinically relevant infections occur in the interstitial space fluid and the distribution and time course of the antibiotics into the tissues is possibly different from the pharmacokinetics in blood. Consequently the target site pharmacokinetic parameters of antibiotics gain increasing attention.

We have therefore measured the target site pharmacokinetics of several antibiotics (Cefpirome, Fosfomycin, Meropenem, Piperacillin) in patients with severe septic infections using microdialysis. The local ethics committee (in accordance with the Declaration of Helsinki and the Good Clinical Guidelines of the European Commission) at the Medical Faculty of the University of Graz approved the studies. Microdialysis probes were inserted during surgery or under CT guidance into inflamed tissue and into non-inflamed tissue for comparison. Probes were calibrated using the retrodialysis method and after administration of the antibiotic under test samples of the microdialysate were collected in 20 minute intervals together with corresponding plasma samples.

The results show that tissue concentrations of the investigated antibiotics can be markedly different from their corresponding plasma concentrations and that there are marked differences in the concentrations between inflamed and healthy tissues.

Target site pharmacokinetic data are therefore a valuable tool in the rational planning of adequate antibiotic dosage regimens in severe infections.

P-12

I. Domán, T. Illés, P. Than, D. Lőrinczy

THERMAL ANALYSIS OF THE HUMAN INTERVERTEBRAL DISC

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Introduction. Intervertebral disc (IVD) degeneration is a common orthopaedic disorder with significant social and economic impact. The degenerative processes begin early in adulthood and progress thereafter. The pathogenesis of IVD degeneration in humans has been the subject of ongoing research; the basic biomechanical and biochemical alterations had been already described. A new approach to the problem is differential scanning calorimetry (DSC) which has never been applied for the investigation of IVD.

Ojectives. 1.Introducing the application of DSC in the research of IVD degeneration. 2.Setting up of calorimetric standards of the normal ad degenerated components of IVD. 3.Demonstrating differences in the samples of normal and degenerated conditions. 4.Establish the calorimetric standards of the different stages along the degeneration.

Materials and methods. IVD specimens were obtained from L4-L5 segments of cadavers with age ranging from 14 to 86. According to the visual evaluation of macroscopic changes affecting the motion segments (Modic classification) the discs were enrolled into different stages. Following samples preparation calorimetric measurements were carried out by a SETARAM Micro DSC-II calorimeter (SETARAM, France). Paired Student's *t*-test were used for the statistical analysis with a significance level of 0.05.

Results. The components of the discs (anulus fibrosus - AF and nucleus pulposus – NP) showed thermodynamically distinct behavior. In thermal denaturation of normal AF and NP were almost identical regarding the main transition temperatures, but completely different in the total calorimetric enthalpy changes. The thermal denaturation of highly degenerated AF and NP demonstarated significant differences in both the main transition temperatures and the total calorimetric enthalpy changes. The comparison of the calorimetric curves of the thermal denaturation of degenerated and healthy specimens demonstarated significant differences. The results of the DSC measurements of distinct stages of IVD degeneration were highly diverse. Comparing the consecutive stages, highly significant differences were found between stages I, III, and V in both the main transition temperature and the total calorimetric enthalpy changes ($p<0.05$).

Conclusion. According to the present study, DSC proved to be a suitable method for the demonstration of thermal consequences of local and global conformational changes in the structure of the human intervertebral discs. The results clearly proved that definitive differences are present between the components of the discs and the stages of disc degeneration in calorimetric measures.

P-13

U. Eder, M. Butter, E. Zirbisegger, L. Reinsberger, F. Bucar, R. Wintersteiger, H. Juan

IN VITRO TESTING OF SEVERAL STYRIAN WINES FOR POSSIBLE VASOACTIVITY VIA TISSUE CONTRACTION EXPERIMENTS

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Epidemiological studies indicate an inverse relationship between moderate wine consumption and the incidence of coronary heart disease. The beneficial cardiovascular effects of wine - especially red wine - have been attributed primarily to the high content of phenolic compounds and alcohol per se. Numerous studies are focused on the influence of red wine and red wine phenols on cardiovascular function in vivo and in vitro.

In the current study Styrian white wines in comparison to Zweigelt - the most cultivated Styrian red wine - as well as their corresponding grape skin extracts were tested for possible vascular effects on bovine coronary arteries. Therefore the bovine coronary artery strips were attached to a force transducer connected to a bridge amplifier and isometric force was recorded on a multipen recorder. Pre-incubation of the vessels with the white wine varieties (330 µl/ml) and the corresponding aqueous grape skin extracts (20 mg/ml; wet weight) inhibited the constrictions to histamine in an NO – dependent manner variably in the range of 5-80% and 40-80%, respectively. By comparison Zweigelt wine and grape skin extract exhibited an inhibition of 53±8% and 74,0±8,2%, respectively.

To characterize the vasoactive component(s) in Styrian grape varieties the Traminer grape skin extract was separated representatively using several methods (ultrafiltration, SPE on Isolute C18(EC) and Isolute SCX-2, CC on Sephadex G-10 and polyamide). The resulting fractions were subsequently bioassayed for vasorelaxing activity using the same test-system. The preliminary results give evidence of a very hydrophilic, non-phenolic positively charged basic compound with a molecular weight below 1000 Dalton.

Our findings demonstrate that certain Styrian wines have remarkable coronary artery dilating effects in vitro and that also non-phenolic components in grapes may contribute to cardiovascular protection. Further separation steps as well as phytochemical and pharmacological investigations are in progress.

P-14

Sz. Fejős, A. Keszthelyi, D. Lőrinczy

THERMOANALYTICAL INVESTIGATION OF COCOA BUTTER AND MILK CHOCOLATE

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The cocoa butter as a main constituent plays an important role in the chocolate manufacturing. It is responsible for the taste, melting and crystallization temperature as well as for the delight value of chocolate. Any change in the sort or weight percentage of cocoa butter will change the characteristics and all other parameters of the chocolate. Environmental factors such as the change of storage temperature can cause some recrystallization which results in the loosening of consumption enjoy.

Experiments were performed and data will be presented on the effect of repeated heating and cooling on the change of melting and crystallization temperature both for cocoa butter and milk chocolate. We have looked for the effect of heating rate in this process too. We made experiments for the crystallization kinetics: the samples were heated with 1.2 K/min scanning rate in 0-100 ºC temperature range and cooled to the starting temperature of crystallization with the same way and held isotherm at this temperature up to the end of crystallization. This process was repeated by changing the isotherm temperature with 1 ºC up to the final temperature of crystallization process.

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J. Gardi, J. M. Krueger, R. C. Speth

PREPARATION AND A SIMPLE PURIFICATION OF ^{125}I-LABELED RAT GHRELIN BY HPLC

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Ghrelin is an acylated peptide displaying growth hormone-releasing activity. It is predominantly produced by the stomach, with substantially lower amounts derived from other tissues. The purified peptide consist of 28 amino acids in which the serine 3 residue is n-octanoylated. The sequences of rat and human ghrelin differ by only two amino acid substitution. Ghrelin is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R). GHS-Rs are concentrated in the hypothalamus-pituitary unit but also distributed other central and peripheral tissues. We show a simple, alternative method to prepare the rat [^{125}I-His^{9}]-ghrelin.

Iodogen was used for radioiodination of rat ghrelin. Sodium phosphate buffer (500mM, pH=8.0), synthetic rat ghrelin, Na^{125}I were added into a iodogen tube successively. The labeling reaction was terminated by addition of $Na_2S_2O_5$ after 10 minutes. KI was then added as a carrier for the ^{125}I to minimize the amount of ^{125}I that is retained on the high performane liquid chromatography (HPLC) column. The mixture was loaded onto the chromatographic column. The HPLC comprised a single isocratic system connected to a flow-through UV detector capable of monitoring peptide bonds at 210 nm and to a flow-through radioiodine detector. The separation was carried out on a C_{18} column (0.46 x 25 cm, 100Å). The mobile phase as 24.5 % acetonitrile and 75.5 % triethylamine phosphate (83 mM phosphate, pH=3.0). The labeled peptide was eluted isocratically at a flow rate of 2 ml/min. The major radioactive peak contains the putative rat [^{125}I-His^{9}]-ghrelin. A smaller peak elutes later, probably representing the diiodinated peptide. The concentration of rat [^{125}I-His^{9}]-ghrelin is approximately 200 nM in the saved fraction.

This radioiodinated peptide could be used for radioimmunoassays to determine ghrelin levels in biological samples and as a radioligand in binding assays to study the physiological roles of ghrelin.

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P-16

C. Gatschelhofer, C. Magnes, M. R. Buchmeiser, F. Stelzer, T. R. Pieber, F. M. Sinner

MONOLITHIC STATIONARY PHASES PREPARED BY RING-OPENING METATHESIS POLYMERISATION: INFLUENCE OF DIFFERENT POLYMERISATION PARAMETERS ON SEPARATION PERFORMANCE

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Miniaturisation is a challenging requirement in the field of proteomics and drug discovery. Therefore, separation media with smaller inner diameter and higher separation performance are needed [1]. Monolithic columns are one attempt to reach this goal. The main advantage of monolithic columns is that they provide lower back-pressure in combination with enhanced diffusional mass transport, leading to shorter separation times. Futhermore, they do not require time-consuming packing procedures or the manufacturing of end frits.

Monolithic capillary columns were prepared from silanised fused-silica capillaries of 200 µm inner diameter by Ring-Opening Metathesis Polymerisation (ROMP) [2]. Monolithic columns based on norborn-2-ene (NBE) and 1,4,4a,5,8,8a-hexahydro-1,4,5,8,-exo,endo-dimethanonaphtalene (DMN-H_6) are copolymerised with Grubbs-type initiator $Cl_2(PCy_3)_2Ru$=CHPh using suitable porogenic systems (toluene, 2-propanol) [3].

The synthesised monoliths allow rapid and highly efficient separation of proteins and peptides by reversed-phase high-performance liquid chromatography.

We report the preparation of monolithic capillaries with changing polymerisation parameters (ratio DMN-H_6/NBE to porogen, Ph_3P concentration) and the influence on the formed structure. The effect of the preparation procedure separation performance will be shown by different peptide and protein mixtures.

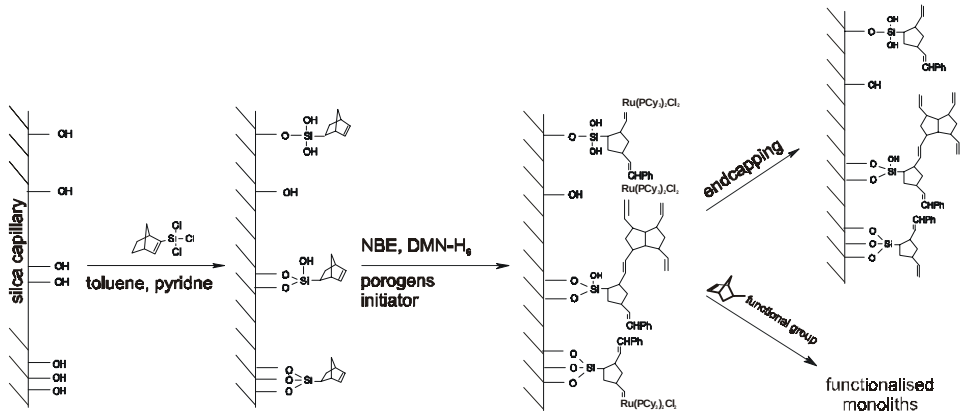

Figure 1: Synthetic route for the preparation of monoliths

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P-17

M. Gfrerer, D. Bandoniene, E. Lankmayr

SELECTIVE EXTRACTION OF PAHS FROM PUMPKIN SEEDS USING ACCELERATED SOLVENT EXTRACTION

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Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants of both anthropogenic and natural origin. Due to their ubiquitous distribution, lipophility and stability PAHs accumulate in fatty tissues and, therefore, in food chains. In general, the first step in the analysis of PAH-contaminated fatty matrices is a solid-liquid extraction using more or less apolar solvents. As a matter of fact, fatty materials like fatty acids, fatty acid esters, lipids or triglycerides are co-extracted and, in most cases, interfere with the chromatographic analysis. It is common analytical practice to perform some steps of clean-up to remove the co-extracted lipids from such samples prior to analysis. Clean-up procedures can include size exclusion chromatography (SEC), gel permeation chromatography (GPC), acid treatment, methanolic alkaline hydroxide saponification, adsorption chromatography and combinations of several separation methods. These procedures add time to the sample preparation, increase the potential for analyte losses or sample contamination and, additionally, require high purity chemicals and solvents. As an alternative, a selective extraction procedure could produce lipid-free extracts. Accelerated solvent extraction (ASE), in contrast to all other extraction methods available, uses completely filled extraction cells without an intermixing of the solid bed during extraction. This principle was investigated in the presented study for the selective extraction of PAHs from pumpkin seeds, a material containing about 40 percent fat and lipids. The extraction cells were filled with different amounts of silica gel, filter agent (diatomite) and sample and were extracted with cyclohexane or n-hexane/acetone-mixtures. The results are comparable with those from a conventional extraction-saponification-adsorption chromatography-method [1].

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P-18

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DDT-DEGRADATION DURING SAMPLE PREPARATION: A CONSIDERATION

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From the 1940s the insecticide p,p'-DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane was heavily used in agriculture as well as for the control the spread of vectorborne human deseases, specially typhus and malaria. Due to its persistence and lipophilic nature, DDT tends to bioaccumulate in flora and fauna, and was, therefore, banned in many countries in the early 1970s. Since the ratio of DDT and its main metabolites can be used to infer inputs of old versus new DDT residues or to attempt to characterize the importance of various aspects of endocrine disruption, with the degradation products more effective than the parent DDT, an accurate analysis of the DDT species is mandatory.

As Foreman & Gates reported [1], DDT is known to be fragile at high temperatures and shows matrix enhanced degradation during GC injection. In general, similar parameters – high temperatures and/or pressure and a catalytic surface – are applied during modern solid-liquid extraction methods like microwave-assisted extraction (MAE), fluidized-bed extraction (FBE) and accelerated-solvent extraction (ASE).

The presented study shows that DDT can break down during enhanced solid-liquid extractions comparable to the degradation process during GC-injection. Additionally, the benefit of isotope-labelled DDT as internal standard is demonstrated.

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P-19

R. E. Gyurcsányi, G. Nagy, E. Lindner, M. R. Neuman

AMPEROMETRIC MICROCELLS FOR ENZYME ASSAY

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Enzymes are normally concentrated in cells and tissues where they perform their catalytic function. In disease, however, certain enzymes tend to leak into the circulation. More than 50 enzymes have been found in human serum and many of them are the subjects of routine clinical analysis. In average clinical laboratories up to 25% of the work are diagnostic enzyme assays. Microfabricated electrochemical devices provide numerous advantages associated with miniaturization and cost effective mass production. Therefore, thin-film photolithography and thick-film screen-printing, has been utilized in our laboratory to fabricate different designs of planar amperometric microcells for enzyme assays in a few microliter samples. The devices and methodologies for the determination of putrescine oxidase (PUO), proline iminopeptidase (PIP) and alkaline phosphatase (ALP) enzyme activities are summarized with the performance characteristics of the assays. The determination of PIP and PUO activities are important in the diagnosis of bacterial vaginosis, a lower reproductive track infection, and the premature rupture of the amniotic membrane, respectively. The alkaline phosphatase enzyme is intensively used as active label in immunoblotting and enzyme immunoassays (EIA). In the clinical practice high level of ALP were correlated with Paget`s disease (inflammation of the bone), osteomalacia (softening of the bone), hepatitis and obstructive jaundice and lower than normal levels with hypophosphatasia.

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P-20

M. Hölbling, G. Gübitz and M. G. Schmid

RAPID ENANTIOSEPARATION OF GLYCYL- AND DIASTEREOMERIC DIPEPTIDES USING MICRO-HPLC AND TEICOPLANIN

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Chiral separation has turned out to be an important tool in analytic purpose. It is well established that about 40 % of the drugs in use are chiral and that the pharmacological activity is mostly restricted to one of the enantiomers. Thus, the development of methods for enantiomer separation for controlling synthesis, for enantiomeric purity check and for pharmacodynamic studies is attracting increasing interest.

Dipeptides are basic and essential components in biological systems either as individual compounds or as constituents of proteins. D-amino acids have been recently shown to play a certain role in human physiology. Therefore, the development of analytical methods for the enantioseparation of dipeptides is an important tool for investigations of peptides and proteins.

This study is about chiral separation of both glycyl- and diastereomeric dipeptides by micro-HPLC. As a stationary phase, teicoplanin, chemically bonded to 3.5 µm silica gel was used. This material was packed into 10 to 20 cm columns of 1 mm ID. A home-built micro-HPLC-system consisting of a normal HPLC pump with a passive split, a micro injection valve (200 nL) and a conventional UV-detector with a micro flow-cell was used. The advantages of such a miniaturized system are less solvent-, chiral stationary phase- and sample consumptions. Teicoplanin, a macrocyclic antibiotic, on glycopeptide basis, shows high enantioselectivity as chiral selector for acidic, basic and neutral compounds. Chiral recognition takes place through inclusion in pockets of the macrocyclic chiral selector; moreover, hydrogen bondings, dipole-dipole interactions, hydrophobic interactions, and π–π interactions are to be taken into account.

A broad spectrum of dipeptides was resolved with this system, whereby reversed phase mode with different organic modifiers such as methanol, ethanol and acetonitrile was used.

P-21

B. Jakab, D. Reglődi, R. Józsa, T. Hollósy, A. Tamás, A. Lubics, I. Lengvári, J. Szolcsányi, J. Németh

DISTRIBUTION OF PACAP-38 IN THE CENTRAL NERVOUS SYSTEM OF VARIOUS SPECIES DETERMINED BY A NOVEL RADIOIMMUNOASSAY

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a member of the vasoactive intestinal polypeptide (VIP)/secretin/glucagon peptide family. This biologically active neuropeptide occurs in two molecular forms: PACAP-38 and PACAP-27. Ovine PACAP-38 was first isolated from hypothalamus by its potent activity in stimulating cyclic AMP production in cultured rat pituitary cells. Soon after the isolation and chemical characterization of PACAP, the first radioimmunoassay (RIA) methods have been developed, but it is still a relatively rarely used laboratory technique in the field of PACAP research. The aim of the present study was to develop a new, highly specific PACAP-38 assay, and using this RIA to determine quantitative distribution of PACAP-38 in the central nervous system of various vertebrate species applying same technical and experimetal conditions.

Description of PACAP-38 specific radioimmunoassay: *Antiserum:* "88111-3" was raised against a conjugate of Cys^{23}-PACAP 24-38 and bovine thyroglobulin coupled by carbodiimide in rabbit. *Tracer:* mono-^{125}I-labelled ovine PACAP 24-38 C-terminal fragment was prepared in our laboratory. *Standard:* ovine PACAP-38 was used as a RIA standard ranging from 0 to 1000 fmol/ml. *Buffer:* the assay was prepared in 1 ml 0.05 mol/l (pH 7.4) phosphate buffer containing 0.1 mol/l sodium chloride, 0.25 % (w/v) BSA and 0.05 % (w/v) sodium azide. *Assay procedure:* 100 µl antiserum (working dilution 1:10000), 100 µl RIA tracer (5000 cpm/tube) and 100 µl PACAP-38 standard or unknown samples were measured into polypropylene tubes with the assay buffer. After 48-72 h incubation at 4 °C, the antibody-bound peptide was separated from the free one by addition of 100 µl separating solution (10 g charcoal, 1 g dextran and 0.5 g commercial fat-free milk powder in 100 ml distilled water). Following centrifugation (3000 rpm, 4 °C, 15 min) the tubes were gently decanted and the radioactivity of the precipitates was measured in a gamma counter. PACAP-38 concentrations of the unknown samples were read from a calibration curve.

Different areas of the brain (brainstem, diencephalon, telencephalon) and the spinal cord were removed from rats, chickens and fishes. Tissues were weighed and homogenized in ice-cold distilled water. The homogenate was centrifuged and the supernatant was transferred for PACAP-38 radioimmunoassay. The PACAP-38 contents of tissues were expressed as fmol/mg wet tissue weight.

Our results show that the antiserum (88111-3) used in the RIA turned to be C-terminal specific, without affinity to other members of the VIP/secretin/glucagon peptide family. The average ID_{50} value was 48.6 ± 3.4 fmol/ml determined in ten consecutive assays. Detection limit for ovine PACAP-38 proved to be 2 fmol/ml. PACAP-38 immunoreactivity was present in the examined brain areas of each species studied, with highest concentration in the rat diencephalon. High levels of PACAP-38 were detected also in the rat telencephalon, followed by spinal cord and brainstem. The central nervous system of the fish also contained considerable concentrations of PACAP-38, while lowest concentrations were measured in the central nervous system of the chicken.

P-22

G. Keresztury, S. Holly and T. Lóránd

ANALYSIS OF THE VIBRATIONAL SPECTRA OF SOME NEW *E*- AND *Z*-4-ARYLIDENE-3-ISOCHROMANONES – PART 2

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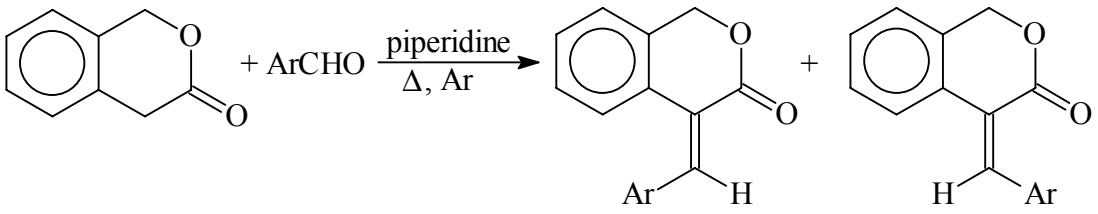
Recently we have published the solvent-free synthesis of some (*E)*- and (*Z)*-4-arylmethylene-3-isochromanones [1]. These compounds were prepared by base catalyzed Knoevenagel condensation. The reaction is usually stereospecific, providing either the *E*- or the *Z*-isomer, but depending on the aromatic aldehyde, it may occasionally yield a mixture of the two isomers that can be separated by means of column chromatography. Thus in the case of 2-nitrobenzaldehyde a mixture of *E*- and *Z*-isomers formed (**1E** and **1Z**), while the pyrrol-2-carbaldehyde furnished only the *Z*-isomer (**2Z**).



E-series

1E (Ar = 2'-$O_2NC_6H_4$)

Z-series

1Z (Ar = 2'-$O_2NC_6H_4$)

2Z (2'-pyrrolyl)

Vibrational spectroscopy is known to be sensitive to conformational changes and structural differences between the E- and Z-isomers; however, the empirical interpretation of the concomitant spectral differences in molecules as large as isochromanones is far from being straightforward. This has been shown in a recent analysis of the infrared and Raman spectra of the 2'-furyl-substituted derivative [2] for which our DFT-based quantum chemical calculations gave four possible conformers. The corresponding *simulated infrared spectra* obtained by the use of scaled quantum mechanical force fields and their comparison with the measured IR spectra (in solid, solution and vapor phases) could be used to assign the spectra to specific isomers and verify the assignment of characteristic spectral features observed.

As a continuation of this vibrational analysis, the IR and Raman spectra of the practically feasible isomers of 2'-pyrrolyl (**2Z**) and 2'-nitrophenyl derivatives (**1E** and **1Z**) were studied with the aim of investigating the steric effects and/or H-bond interactions influencing the stabilization of certain isomers and conformers, and arriving at a detailed understanding of the fine spectral details.

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P-23

J. Kiss, K. Kiss, M. Červinka, E. Rudolf, J. Szeberényi

SODIUM SALICYLATE DECREASES THE VIABILITY OF PC12 CELLS VIA INHIBITION OF THE NFκB TRANSCRIPTION FACTOR

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Sodium salicylate is a strong analgetic and anti-inflammatory drug. In therapeutic concentration it has an inhibitory effect on the cyclooxigenase enzymes (COX1, COX2). Besides this effect it influences the level of other signal transduction proteins including NFκB (nuclear factor kappa B).

The NFκB transcription factor is present in most cell types, it is a general transcription factor which is activated by several stimuli and can trigger either apoptosis or cell survival. It can be found constitutively in the cytoplasm bound to an inhibitory protein, IκB (inhibitory kappa B). During its activation this IκB is degraded and the free NFκB translocates to the nucleus. Its binding to the consensus sequence of the DNA regulates the transcription of different genes. Sodium salicylate inhibits the degradation of IκB, thus the NFκB activation is cannot occur. According to publications inhibition of this activation leads to programmed cell death, apoptosis.

The main goal of this study was to demonstrate that inhibition of the NFκB decreases the viability of PC12 cells and investigate the nature of the cell damage and death.

For our experiments we treated the cells with different concentrations of sodium salicylate in the presence or absence of other antiapoptotic agents (NGF - nerve growth factor, dbcAMP- dibutyril cyclic AMP). The assesment was done by a direct counting of cells in Bürker chamber or by WST-1 assay. We demonstrated the decreased NFκB activation by electrophoretic mobility shift assay, too. For detection of cell death we used DNA fragmentation assay and time-lapse videomicroscopy and took microscopic pictures.

Our results show that sodium salicylate decreases the number of living cells in dose-dependent manner and causes apoptosis in high concentration (10-20 mM). Treated cells were undergoing apoptosis as seen on our records detected with time-lapse videomicroscopy (formation of blebs, later apoptotic bodies) as well as the result of the DNA fragmentation assay showed. The decreased NFκB activation level showed that the inhibition of NFκB could cause these effects. In our further experiments we would like to perform immuncytochemistry for observing the NFκB translocation and prepare Western blot studies to observe the protein expression whose transcriptions are regulated by NFκB.

P-24

J. Kiss, P. Engelmann, V. Csöngei, P. Németh

THE EFFECT OF EARTHWORM COELOMIC CELLS ON VIABILITY OF MAMMALIAN CELL LINES

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Earthworm is a good model organism for studying innate immunity. It protects itself against the environmental pathogens by physical-chemical barrier and biologically active coelomic fluid. The coelomic fluid contains proteins, are responsible for hemolytic, hemagglutinating, antibacterial, antifungal, and cytotoxic effects and it also contains effector cells (coelomocytes). These cells participate in phagocytosis, encapsulation, granule formation and presumably production of antibacterial and cytotoxic proteins as well.

In our previous study we detected the antibacterial effect of the coelomic cells. The main goal of the recent experiment was to observe the cytotoxic effect of coelomocytes. Our aim was to prove that the lytic proteins, which can be found in the coelomic fluid (fetidin, lysenin, eiseniapore, haemolysins, CCF- coelomic cytolytic factor- and lysozyme) are a secreted product or storage place of coelomocytes. In our experiments used cell-free coelomic fluid or supernatants of short-term cultured coelomocytes and lysates, following by mechanical and detergent extraction of coelomocytes. These compounds were tested on different mammalian cell lines (HeLa, HEp2 and PC12). We made experiments using whole or treated (Proteinase K, trypsin, heat-inactivated) coelomocyte lysates. As controls we used mammalian cell lines and applied in the same way like coelomocyte lysates. In every case we made the viability assessment by photometrical measuring using MTT or WST-1 assay and took microscopic pictures.

Our results show that the coelomic fluid, the supernatant and the coelomocyte lysate (CCL) decreased the number of the living cells in comparison with the controls. The presence of cytotoxicity was supported by the phase-contrast microscopic pictures, in which we could immediately observe the vacualisation of the cytoplasm and the swelling of the nuclei. Treated CCLs showed that cytotoxic molecules presence in coelomocytes can be proteins. Boiling and Proteinase K digestion inactivate the effect of the coelomic cell lysate, but the trypsin digestion is not yield the inhibition of cytotoxicity.

In this study we characterized the coelomocytes with cytotoxic activity. This effect belongs to the cells however similar effects are present in the coelomic fluid. Several lytic molecules were detected in coelomic fluid but it is not described clearly which are released from the coelomocytes.

P-25

I. Klimant, C. Moser

A NEW ENCODING CONCEPT FOR LUMINESCENT MICROSPHERES USED IN RANDOMLY ORDERED SENSOR ARRAYS

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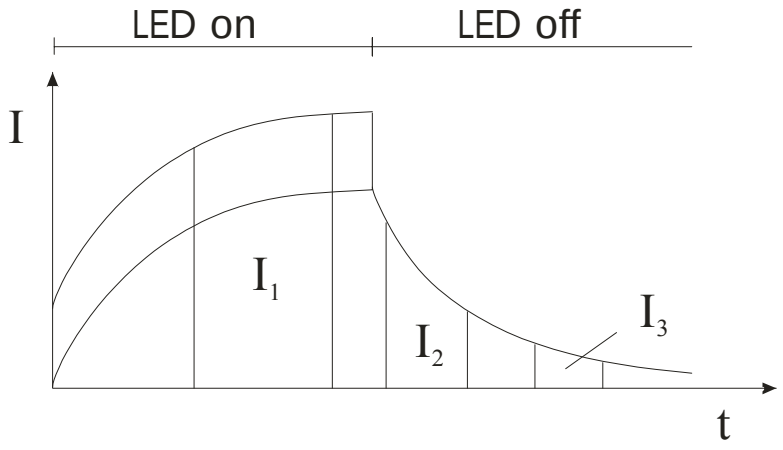
Fluorescence encoded micro particles are a powerful tool in multiplexed immuno- and oligonucleotide analysis. Recently described approaches are randomly ordered high- and low-density sensor arrays and flow cytometry. Another field of application for coded particles is combinatorial chemistry.

Encoding of polymer particles can be performed by staining them with fluorophores with distinguishable spectral properties or by mixing two or more of them at different ratios resulting in defined pattern of fluorescence. Recently a new scheme introduced the use of phosphorescence decay time as an additional parameter to distinguish individual sets of nanospheres [1]. In all approaches different spectral windows are required to identify the nature of individual particles.

Here we present and characterise new encoded luminescent polymeric microspheres, specially optimised for their use in randomly ordered sensor arrays. It allows the use of a conventional fluorescence microscope equipped with a triggered blue light emitting diode as light source and a tuned fast triggered CCD-camera. No filter change is necessary during identification. The particles were stained with a couple of luminescent dyes showing distinguishable luminescence decay but similar (optimally identical) spectral properties. A phosphorescent ruthenium(II)-polypyridyl complex (selected from a series of complexes with almost identical spectral properties but different decay times ranging from 1µs to 6 µs) and a fluorophor with a nanosecond decay were mixed in defined ratios. One fluorescence image (I1) is recorded during the excitation (LED on) – quantifying the overall signal of both dyes - and two phosphorescence images (I2 and I3) are recorded during the decay of the phosphorescence dye (LED off). From these 3 images the identification of the phosphor (I2/I3), its concentration (I2) and the ratio of fluorophor and phosphor (I1/2) is possible. Adding the size of particles as additional code, allows distinguishing more than 1,000 individual particle populations.



P-26

J. Koidl, N. Grobuschek, M. G. Schmid, G. Gübitz

ENANTIOSEPARATION OF AMINO ACIDS AND VARIOUS DRUGS BY CAPILLARY ELECTROCHROMATOGRAPHY AND MICRO-HPLC USING A TEICOPLANIN AGLYCONE STATIONARY PHASE

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Chiral separation of amino acids and pharmaceuticals is performed by capillary electrochromatography (CEC) and micro-HPLC on a teicoplanin aglycone stationary phase. Teicoplanin, a macrocyclic antibiotic, on glycopeptide basis, shows high enantioselectivity as chiral selector for acidic, basic and neutral compounds. It is used as chiral stationary phase (CSP) in HPLC, as buffer additive in capillary electrophoresis and recently as CSP also in CEC. A new CSP based on teicoplanin aglycone, which lacks of the substituted D-glucosamine and D-mannose moieties, was found to show different separation behavior in HPLC compared to teicoplanin.

We used a capillary packed with teicoplanin aglycone bonded to silica gel 3.5 μm for CEC and micro-HPLC investigations [1]. This CSP showed enantioselectivity for different drugs, such as ß-blockers, sympathomimetics, calcium antagonists and diuretics. In particular remarkable chiral separation of amino acids was achieved. Separations were carried out comparing reversed phase mode and polar organic mode. Whereas for amino acids the reversed phase mode was found to be superior, for drugs better results were obtained with the polar organic mode.

[1] Enantioseparation of amino acids and drugs by CEC, pressure supported CEC and micro-HPLC using a teicoplanin aglycone stationary phase

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P-27

T. Konecsni, F. Kilár

STUDY OF THE LABELING OF HUMAN SERUM TRANSFERRIN USING FITC

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Fluorescently labeled proteins are used in wide range to investigate several biological problems. There are some cases when labeled proteins are not commercially available and the researcher should make the labeling. Most of the fluorescent probes react with accessible primer amines exposed on the protein surface. The distribution and accessibility of these amino groups differ from protein to protein. However, general protocols for getting optimally stained conjugate are available. The conditions of labeling should be optimal for keeping the biological activity of the protein. The derivatization depends on several conditions, e.g., pH of the buffer, concentration of protein and dye, the molar excess of dye to protein, etc. Human serum transferrin stained with FITC serves as a good model to investigate the labeling mechanism of amine reactive dyes using different dye to protein ratios because the derivatized protein can be separated well from the excess of FITC by capillary electrophoresis without previous purification step. Iron free transferrin samples in the range of 1.25×10^{-7} M to 1.25×10^{-4} M were labeled in the presence of 10^{-3} M, 10^{-4} M and 10^{-5} M FITC. UV, broadband fluorescent and laser induced fluorescent detection was applied for evaluation. Increasing the degree of labeling the mobility of the labeled protein decreases. The limit of detection of the sample with the highest degree of labeling was 10 ng/ml = 0.125 nM measured with the LIF detector. The lowest protein concentration used in labeling procedures was between the mikro and nanomolar range (10 μg/ml = 0.125 μM).

P-28

C. Korepp, H.J. Santner, K.-C. Möller, J. O. Bessenhard, M. Winter

IN SITU FTIR INVESTIGATION OF THE SEI FORMATION OF ELECTROLYTE ADDITIVES IN LITHIUM ION BATTERIES

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Electrolytes, which are used in conventional lithium ion batteries, work far beyond their thermodynamic stability. Electrolyte reduction and oxidation occurs at the interfaces between the organic electrolyte and the negative and positive electrode. Fortunately, the products of electrolyte reduction normally form a protective film, the so called solid electrolyte interface (SEI) on the surface of the negative electrode during the first charge step. This film prevents further electrolyte decomposition throughout the following charge/discharge cycles. In case of electrolyte solvents, which are not able to form such a film, e.g. propylene carbonate, and which tend to solvated intercalation, a film forming compound has to be added. Vinylene additives have proven to form effective films on graphite anodes even when only present in very small amounts in the electrolyte (1 % v/v).

Cyclic voltammetry and charge/discharge experiments are used to verify the capability of the investigated additives and to compare their electrochemical properties. To our opinion the reductive decomposition of vinylene monomers and consequent SEI film formation proceeds according to a polymerization mechanism shown in Fig. 1. In order to better understand the mode of operation of the additives, *in situ* FTIR spectroscopy was applied. A special institute-made spectro-electrochemical cell was developed for measurements at dry and oxygen-free conditions. The FTIR studies give strong evidence that the vinylene additives, added to a 0.5 M $LiClO_4$/PC electrolyte are decomposed via a reduction of the double bond. When a glassy carbon working electrode is polarized at the reduction potential of the electrolyte, significant changes of characteristic IR bands can be observed. Results of different vinylene additives will be presented, similarities and differences of the investigated additives will be highlighted.

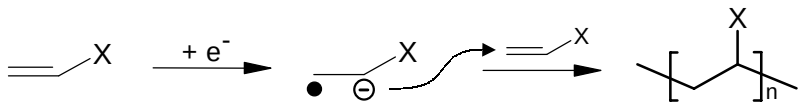

Figure1: Cathodic, i.e., by reduction induced polymerization of vinylene monomers

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DETERMINATION OF FLAVONOIDS, CHLOROGENIC ACID AND CAFFEIC ACID IN APPLE AND PEAR SAMPLES USING HPLC

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In this study the main phenolic compounds were determined by HPLC from the fruit skin of three apple and two pear cultivars. All samples were collected during summer and autumn in 2002, every 3 weeks. The main objective was to find the best conditions for the separation of quercetin and quercetin-3-O-glycosides, caffeic and chlorogenic acids, and determine how their concentration changes in the apple and pear samples.

Chlorogenic acid, caffeic acid, quercetin and quercitrin were found in all three apple cultivars. Rutin wasn't found, and hyperoside was present only in Jonathan apple. The amount of chlorogenic acid, caffeic acid and quercetin during the experiment was between 0-0,1 mg/g dw, and the changing of the dry weight was not significant. In Golden Spur the presence of the above phenolic compounds was higher in the first two samples than later, while in Jonathan apple there was a sudden increase in dry weight in October. Quercetin showed lower amount than the quercetin-glicosides (quercitrin and hyperoside). Quercitrin was the highest amount component. After the higher level of this glycoside at the beginning, there was a decrease in Idared and Golden Spur apples, whereas a continuous increase could be observed in Jonathan. Following an increase during the summer period, the amount of quercitrin decreased again in September in each apple cultivar.

Chlorogenic acid, caffeic acid quercetin and quercitrin were found in Williams and Bosckobak pears, but no rutin and hyperoside. The amount of chlorogenic acid increased between the first and the last sample collection. In August there was a sudden decrease in both pears. The amount of caffeic acid and quercetin did not change significantly during the collection period, but in Bosckobak the dry weight of quercetin was higher almost ten times (0,8-0,9 mg/g dw) than in Williams (0,1-0,2 mg/g dw). The amount of quercetin was smaller than that of its glycoside, quercitrin. Quercitrin was the greatest amount component, similarly to apple. The curve of quercitrin is similar in the two pear cultivars, its amount increased until mid-summer, then decreased in late summer, early September, and finally increased again in the second half of September.

P-30

B. Kovács, Á. Markovics, G. Nagy

ANODIZED ALUMINA AS SUPPORT FOR REFLECTION BASED OPTICAL AMMONIA SENSOR

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Optical chemical sensors became an alternative to electrochemical sensors for sensing ionic substances, pH as well as gases such as oxygen, ammonia or carbon dioxide. Fluorescence as well as reflectance based sensors are mainly used where contactless or long range sensing is required.

The active substances involved in the optical sensing process usually are applied in sensors dissolved in a polymeric matrix. For this purpose a wide variety of materials, such as plasticized PVC, silicones, polyurethanes, cellulose derivatives are often used. Several methods are used to immobilize the dye and the other components of the sensor: binding them covalently to the polymer matrix, using lipophylic indicator or making them soluble by ion-pairing.

Here, we report on a reflection measurement based optical ammonia gas sensor with a sensitivity in the ppb range with a response time of some seconds. A dust free alumina sheet was anodized in sulfuric acid and then immersed into a dye (bromophenol blue) containing solution. A highly reflective, yellow colored surface was obtained this way, where the indicator dye was entrapped into the pores of the aluminum oxide layer. After the immobilization was completed the pores were filled with different kind of nonpolar solvents to avoid leaching and pH cross-sensitivity. The anodized alumina served as a mechanical support, as a reflecting element and as porous material for the immobilization of the indicator.

Depending on the concentration of ammonia the embedded indicator dye changed its color from yellow to blue, and hence, the attenuation of the reflected light. The maximum attenuations occured at 450 nm (acidic form) and 620 nm (basic form). The sensor was placed into a home-built flow-through-cell. A bifurcated fiber bundle connected to a colorimeter was used to monitor the optical signal change using two-wavelengths-referencing method.

The anodizing and the immobilizing procedures are reported in detailes. Furthermore the dynamic range, signal changes, sensitivities depending on the different filling materials are shown. The forward and reversed response times as well as the storage stability of the sensors are also reported.

P-31

S. Köstler, G. Jakopic, K. Stana-Kleinschek, V. Ribitsch

CHARACTERISATION OF ULTRA-THIN POLYMER LAYERS

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Well defined ultra-thin polymeric layers can be deposited onto a variety of different substrates by the so called layer-by-layer deposition technique. In this approach, single layers of polyelectrolytes are adsorbed from aqueous solution onto a charged polymeric or inorganic substrate. Due to charge reversal on the newly created surface, these layers allow for further adsorption of an oppositely charged polyelectrolyte. Multilayers can be obtained by consecutive adsorption of positively and negatively charged polyelectrolytes. Other substances like e.g. biomolecules, fluorescent dyes, nanoparticles or neutral polymers can be incorporated into these multilayers and make them interesting for e.g. chemo- and biosensors, biointerfaces, and drug delivery, as well as for electronic and photonic applications.

Multilayers of PDADMAC, (poly(diallyldimethylammonium chloride)) and PSS, (poly(sodium 4-styrenesulfonate)) were deposited onto inorganic (silicon, glass) and pretreated polymer (PET, PTFE) substrates. Alkaline surface hydrolysis was chosen as pretreatment for PET and an UV/hydrazine treatment was chosen for PTFE.

The surface properties of these layers were determined by a combination of different surface and thin-film analytical methods. Electrical surface properties were characterised by means of streaming potential measurement. Polyelectrolyte adsorption was indicated by a marked shift of the isoelectric point and the shape of zeta potential vs. pH curves. With certain presumptions, also mean pK_A values of surface groups and surface charge densities can be estimated. Ellipsometry was used in order to determine the layer thickness and optical constants and to proof a regular layer-by-layer growth. Surface roughness values of the polymer layers were determined by atomic force microscopy in tapping mode. On very smooth substrates, roughness values of about 1 nm or less were found and the initial roughness of polymer substrates was not altered upon layer deposition. Surface thermodynamic properties and wetting behaviour were determined by contact angle measurements. Employing a thermodynamic model, we were able to determine apolar, electron donor, and electron acceptor components of the surface free energy. This distinction between the individual free energy components, provides an opportunity to link wetting behaviour with structural information and functional groups. This is indispensable to interpret more complex wetting phenomena due to polymer chain rearrangement, or to predict the affinity of potential adsorbates (e.g. proteins) to the surface.

With the above described combination of methods one can determine the most important properties of ultra-thin polymeric layers with focus on applications such as sensors and biointerfaces.

P-32

S. Kunsági-Máté, B. Lemli, G. Nagy, L. Kollár

ENERGETICS OF THE PROTON TUNNELING CHANNELS RELATED TO THE CYCLIC HYDROGEN BONDS AT LOWER RIM OF CONE CONFORMERS OF METHYL-, THIA- AND OXA-CALIX[4]ARENES

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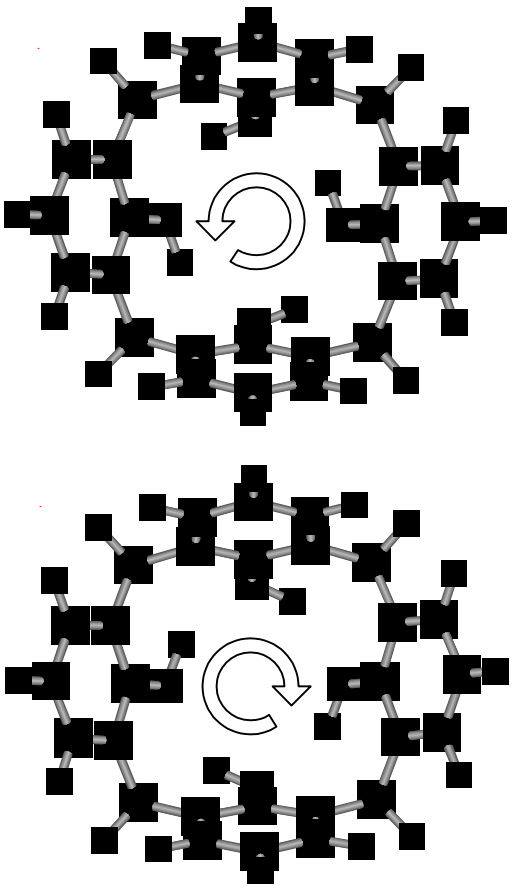
Calixarenes, a family of macrocyclic oligophenols, have attracted much attention because of the simplicity of their skeleton, which is associated with versatile recognition properties both of metallic or organic ions and of neutral molecules. A variety of these compounds belong to the family of calix[4]arenes, where the calix cavity is built from four phenolic units connected by $-CH_2-$, -S- and $-CH_2-O-CH_2-$ bridges at 2,6-positions. The calix[4]arenes have four stable conformers (cone, partial cone, 1,2-alternate and 1,3-alternate). Owing to its finely tunable molecular shape the cone conformer is in the focus of interest in supramolecular chemistry.



In the cone conformer of a calix[4]arene a cyclic hydrogen bond network is formed at the lower rim. This network and the distortion of the bridges between every two neighbouring phenol rings take part in the stabilization of the molecular conformation. These chiral molecules exist in two enantiomeric forms (see the figures). Transitions between these two forms are possible either through exchange of the four protons along their individual hydrogen bonds or by rotations of OH groups around the O-C bonds. Some calixarene derivatives, whose phenolic segments are connected with methylene-, thia- or oxa- bridges, show different cavity sizes, and consequently, also the H – O bond distances are different. Therefore, this series of calixarenes are suitable models to study the dynamic behaviour of protons at the lower rim. In this work we examined the energetics of the proton exchange between the two enantiomers of methylene-, thia- and oxacalix[4]arene derivatives using quantum-chemical methods. The calculations were done with semi-empirical, *ab-initio* and also with density functional methods. The results show that the symmetry of the skeleton plays important role in the transition processes. In a molecule having four-fold symmetry the transitions are taken by tunneling splitting. In contrast, when the symmetry is broken, the exchange is realized by the OH group rotation. The strength of the hydrogen bonds in the cyclic network, and consequently the tunneling effects are highly affected by the length of hydrogen bonds.

P-33

S. Kunsági-Máté, R. Skoda-Földes, L. Szepes, E. Végh and L. Kollár

UNEXPECTED REACTIVITY DIFFERENCE BETWEEN IODO-ALKENE MOIETIES OF STEROIDS POSSESSING REMOTE LACTAME OR CYCLOALKANE STRUCTURAL UNITS: A THEORETICAL APPROACH

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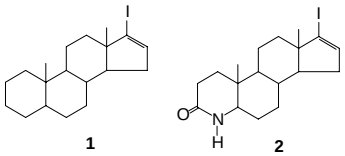
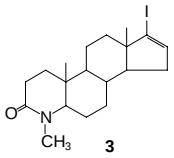
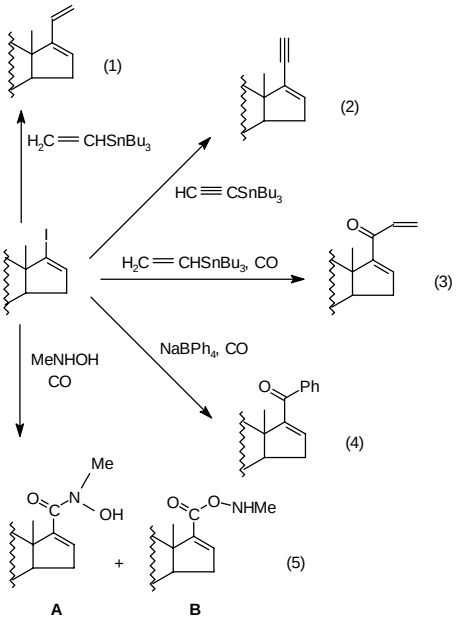
A striking feature of the development of organic chemistry over the past few years has been the better understanding of the consequences of molecular energy distributions often referred as 'long range effects'.[1]







The Stille-coupling of the 17-iodo-16-ene steroidal substrates (see the *Chart* right above) and vinyl-tributylstannane (*Scheme* left, *(1)*[2]) or ethynyl-tributylstannane (*(2)*[2]) resulted in almost quantitative yields of the corresponding diene or enyne, respectively, using **1**, and much lower yields when **2** or **3** were reacted in the presence of $Pd(PPh_3)_4$ as catalytic precursor. Similar reproducible results were obtained in palladium-catalysed carbonylative vinylation (*(3)*[2]) and carbonylative phenylation (*(4)*[3]). The structure of the A-ring seems to influence also the chemoselectivity of the reaction (*(5)*[4]). It is known, that any explanation based on the influence of the structural change in the A-ring on the electronic structure of the far located 'iodo-vinyl' moiety, is very implausible on the basis of the bond structure of these molecules since no conjugated π-systems can be considered in the 'communication' of the two distant parts of the skeleton.

In the present work a newly developed approach [5] explaining the influence of the structural changes in the steroidal A-ring on the reactivity of distal 'vinyl-iodide' moiety in D-ring will be presented. The experimental findings related to the different reactivity of 17-iodo-androst-16-ene derivatives (**1**, **2** and **3**) will be discussed by the structure–activity relation (SAR) analysis of these compounds carried out on the basis of the Rice-Ramsperger-Kassel-Marcus theory. Furthermore, quantum-chemical model calculations will be presented here to focus on the *vibrational energy distribution of the molecules* possessing various A-ring-structures.

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P-34

S. Kunsági-Máté, L. Nagy, G. Nagy, I. Bitter, L. Kollár

INTERACTIONS OF Fe(II) AND Fe(III) IONS WITH THE 'HARD' AND 'SOFT' ION BINDING SITES OF AN OCTAFUNCTIONALIZED *C*-METHYL-CALIX[4]RESORCINARENE DERIVATIVE

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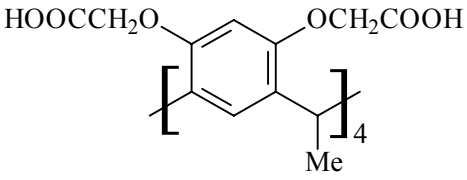

1

The interactions between Fe(II) or Fe(III) ions and aromatic π faces of biomolecules have been shown to play important role in physiological processes.[1] Cation-π interaction-linked sandwich complexes of iron are listed among reversible electrochemical mediators used in amperometric biosensors.[2] So far, great interest has been paid to the complexation studies of these metal ions in aqueous solution.[3] Owing to their polarizability, Fe(II) and Fe(III) ions are considered quite different. The Fe(II) is "softer" Lewis-acid than the relatively "hard" Fe(III) cation. Octasubstituted *C*-methyl-calix[4]resorcinarene (**1**) can form both a "hard" and a "soft" ion binding site with its carboxylate arms and with the aromatic rings, respectively.

In this work, using our earlier successfully employed methodology based on PL measurements and the Job's method, the complex formation of octasubstituted *C*-methyl-calix[4]resorcinarene with both Fe(II) and Fe(III) ions were studied parallel with quantum chemical calculations.

The results show, that in the case of Fe(II) ions as guests, the enthalpy, entropy, and also the Gibbs free energy changes of the four different complexation steps are nearly the same and the **1** - Fe(II) complex with 1:4 stoichiometry is supported at room-temperature. However, the complexation of **1** with Fe(III) ions show quite different thermodynamic behaviour. The 1:1 and 1:2 complex formation results in similar enthalpy-entropy changes, which are higher than the appropriate values found for the Fe(II) ion. Nevertheless, the **1** - Fe(III) complex formation with 1:3 or 1:4 stoichiometry is not supported due to the very low interaction energy derived from these stoichiometries.[4]

In agreement with the experiments, the quantum chemical investigations suggest, that Fe(II) ions seem to form complexes with **1** through cation-π interactions in a favourable way, whilst the complexation of Fe(III) ions is mainly based on the chelate formation with the carboxylate functionalities of the resorcinarene moieties. This observation makes **1** and its analogues promising candidates as special, redox-sensitive host molecules to be used in chemical sensors.

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S. Kunsági-Máté, K. Szabó, I. Bitter, G. Nagy, L. Kollár

COMPLEX FORMATION BETWEEN WATER SOLUBLE CALIXARENE SULFONATE DERIVATIVES WITH C(60) FULLERENE

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Several recent studies have shown that fullerenes exhibit much higher importance than only in chemistry. They also possess some interesting properties related to biological systems. The electron accepting ability of the fullerenes is known for the inhibition of redox enzimes (*e.g.* nitric oxide synthase, thus providing a therapeutic approach for some neurodegenerative disorders[1]) and a large number of fullerene derivatives are competitive inhibitors of the human immunodeficiency virus (HIV) protease.[2] However, the very low solubility of fullerenes in aqueous solvents seems to be the major problem for their biological applications. Two independent works have shown that *p-tert*-butylcalix[8]arene selectively includes the C_{60} fullerene and forms a complexes with 1:1 stoichiometry.[3,4] Soon after efforts have been invested to study the supramolecular complexes of fullerenes with a variety of host systems and also, several strategies have been developed to overcome the natural repulsion of fullerenes for water. The next problem appeared is that most of calixarene-fullerene complexes exist only in the solid state. In most solvents they were either dissociated into each components or precipitated from the solution. Therefore, any spectroscopic proofs for the existence of these complexes could not be found.

In our recent papers,[5-7] the complexation behaviour and the factors controlling the thermodynamic and kinetic stability or selectivity of some calixarene derivatives towards neutral π-electron deficient species also the interaction of a water-soluble calixarene with iron ions[8] were reported. Following our earlier methodology based on PL measurements using the Job's method, in the present work we examine the inclusion complexes of calixarene sulfonate derivatives (1-sulfo-thiacalix[4]arene sodium salt /thiacalix[4]arene-4-sulfonic acid sodium salt/ **1** and 1-sulfo-calix[6]arene /calix[6]arene-4-sulfonic acid sodium salt/ **2**) with C_{60} fullerene. In order to investigate the interaction, 10^{-4} M aqueous solution of **1** (or **2**) and 10^{-4} M toluene solution of fullerene were prepared. For application of Job's method, these solutions were mixed with different molar ratio and the aqueous phase was used for the PL measurements. The results show that **1** and **2** form complexes with the C_{60} fullerene by 2:1 and 1:1 stoichiometry, respectively. Our related quantum-chemical investigations show that C_{60} fullerene is included in a cavity composed of two **1** half bowls. The C_{60} fullerene ball lying much more deep in the cavity of **2** and the negatively charged sulfonate arms probably inhibit the formation of such a bowl-shaped capsule than was found in case of **1**.

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S. Kunsági-Máté, E. Végh, G. Nagy, L. Kollár

INFLUENCE OF THE MOLECULAR ENVIRONMENT ON THE THREE-CENTER VERSUS FOUR-CENTER ELIMINATION OF HYDROGENE-HALIDE MOLECULES FROM HALOGENATED ETHYLENE

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The dissociation reactions of halogenated ethylene (vinyl halides, CH_2CHX, X=F, Cl, Br or I) have been a fruitful testing ground for unimolecular reaction theories. A detailed mechanism of dissociation proceeds via multiple channels. The dissociation mechanism strongly depends on the identity of the halogen substitution, which influences the energetics of dissociation pathways, *i.e*. relative sites of transition states on which the system undergoes during the fragmentation processes. The recent studies of these compounds are particularly interesting because their reaction dynamics induced by photoexcitation can be experimentally investigated by measuring the vibration energy distribution of the dissociated products.[1]

In this work the dynamics of dissociative reactions of vinyl halides was investigated theoretically by Langevin molecular dynamics method in the absence and in the presence of frictional interaction between the molecules and their environment. The results show a redistribution of the vibrational energy on the bonds of vinyl halide molecules under the effect of frictional interaction. This redistribution modify the reaction rates of the dissociation channels. Increasing the frictional coupling between the vinyl halide molecules and their environment, the vibrational energy redistribution increases the transition probability on the four-center elimination channel. This environment-induced change on the rate of reaction pathways demonstrates that the existence of friction may not guarantee statistical behaviour of the dissociation processes of these molecules.[2]

The result derived by Langevin molecular dynamics simulations were analysed on the basis of repulsive or attractive character of the potential energy surface. Since the strength of the carbon-halide bond in the vinyl halide molecules changes with the type of halide atom, the site of the saddle point on the surface will changes resulting either repulsive, either attractive character of the potential surface. The frictional interaction highly depends on the speed of a given atom. In cases of these molecules the carbon-hydrogen bonds have the highest frequencies, therefore they are mostly affected by the friction.

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P-37

S. Landgraf

USE OF ULTRABRIGHT LEDS FOR THE DETERMINATION OF STATIC AND TIME-RESOLVED FLUORESCENCE INFORMATION OF CRUDE OIL SAMPLES

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Ultrabright LEDs are an inexpensive alternative to laser diodes and other short wavelength emitting light sources. They have a high stability, a long lifetime, and a very low power consumption. A large number of publications are already available for fluorescence applications using this type of LEDs. Most of them are describing fluorescence intensity measurements. Only some of them are dealing with time-resolved methods, like single photon timing. LED modulation fluorometry is a very recent application [1] which can also be used for environmental investigations, like the detection of polycyclic aromatic hydrocarbons [2]. Other time-resolved methods applying LEDs are: Time-correlated single photon counting TCSPC/single photon timing (SPT) [3], modulation fluorometry with commercial spectrometers [4] and direct phase measurements [5].

Three different samples have been analysed: Solutions in cyclohexane, contaminated fine and coarse sand. Typical decay times could be found. For the sand samples this range is limited to 10 to 30 MHz due to the high background luminescence from the sand itself. The detection limits estimated from the decay times are 1 to 3 ppm for the solutions (depending on the LED used), 100 ppm for coarse sand, and 2000 ppm for fine sand due to the high absorption of the oil into the sand.

The main application of this technique can be the fast detection of the presence of an oil contamination rather than an analysis of the type of oil. The results clearly indicate that LDs and LEDs are promising light sources for a lot of applications for static and time-resolved fluorescence measurements.

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P-38

S. Lechner, A. Pietzka, E. Katzogiannos, M. Murkovic

ANALYSIS OF MINOR COMPONENTS IN OLIVE OIL

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The world production of olive oil is ca. 2.5 mio t with the major producers Spain, Italy, Greece and Maghrebian countries. As beneficial health effects of the oil were shown (reduction of LDL cholesterol) the main focus of research is on the minor components polyphenols and squalene. In this study the phenols (tyrosol, vanillin, vanillic acid, p-coumaric acid, ferulic acid, luteolin, apigenin, oleuropein) as well as squalene were analysed in dried olives derived from the same tree but from different parts of the plant. Additionally an oil was produced from these fruits with and without the kernels.

Polyphenol analysis: For analysis of the phenols 500 mg of the oil was extracted with 500 µL methanol, centrifuged for 15 min and the extract measured by HPLC. The substances were identified by the use of standard substances comparing the retention times as well as the MS spectra. For HPLC analysis a LiChrospher 100 RP18e 250 x 4 mm was used with water (0.2 % acetic acid) and methanol. The elution gradient was as follows: MeOH increased from 10 to 30 % in 10 min then maintained for 20 min and a stepwise increase of 10 % in 5 min with isocratic elution in between for 5 min each.

Squalene analysis: 10 mg of the oil samples were diluted with 10 ml acetonitril. As eluent (acetone/ acetonitril 50/50 v/v) was used on a LiChrospher 100 RP18e 250 x 4 mm. For detection a RID was used. With this method also a separation of triglycerides is possible. Results for phenolic substances and for squalene will be shown and discussed.

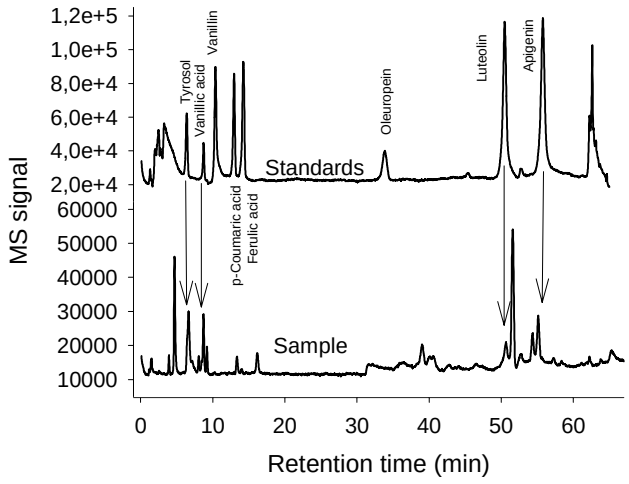

Figure 1: Sample chromatogramm of olive extract

P-39

Litz, B., Szakály, S., Schäffer, B., Lőrinczy, D.

INDICATION OF PROLIFERATION OF LACTIC ACID BACTERIA IN MIXED CULTURES AND ESTIMATION OF THEIR RATIOS BY ISOPERIBOLIC BATCH CALORIMETRY

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The supply and consumption of probiotic foods, and particularly probiotic dairy products, has grown steadily in recent years. In the production of dairy products of this type other microbes must also be used in addition to the microbes which provide the probiotic effect and which generally have a proliferation optimum of 37°C. The reason for this is that probiotic microbes have a neutral taste in dairy products. Consequently, the taste of fermented dairy products is supplied by other microbes. These microbes are likewise lactic acid bacteria, and their proliferation optima are either below (mesophilic) or above (thermophilic) that of the probiotic microbes. It is imperative to have an indication of whether the probiotic bacteria have multiplied at the fermentation temperature used during the technology, since they are what provide the beneficial physiological effect of the product. Isoperibolic batch calorimetry appears a suitable method for indicating this quickly, as the amount of heat released during lactic acid bacterial proliferation differs.

During the experiments we examined the proliferation of butter culture, yoghurt culture, Bifidobacteria, bifidus culture, Lactobacillus helveticus culture, kefir culture, and a probiotic lactic acid bacteria culture which was isolated by ourselves. The examinations were conducted at temperatures used in the production of various dairy products. During the examinations the total plate titer was measured and the isothermic heat flow-time curves of the given cultures were recorded. For the latter approx. 450 mg sterile fat-free milk and approx. 50 mg culture were placed into a mixing batch vessel and left until thermal equilibrium was reached at the given temperature, then the culture was injected into the milk and the heat flow curve of microbe proliferation was recorded for 16 hours by a SETARAM Micro DSC-II calorimeter under isothermic conditions at the given temperature. For determining total plate titer thousand-fold quantities were used, fermentation was conducted until pH 4.7 was reached, then the number of microbes in the culture was determined by internationally standard methods.

In order to analyze the heat flow curves a deconvolutional program was devised which broke them into Gaussian curves, given that the proliferation of individual microbes is log-normal. The Gaussian curve characteristic of the culture was determined, and from the area under the curve the amount of heat released by the creation of one microbe was calculated.

P-40

T. Madl, M. Mittelbach, H. Sterk

ISOLATION AND CHARACTERISATION OF SAPONINS FROM *CHENOPODIUM QUINOA* WILLD.

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The Andean food crop *Chenopodium quinoa* Willd. is known to be rich in saponins. Especially in the pericarp a high concentration of these compounds from 2 up to 6 percent is found.

Therefore this part of the seed was extracted with a water-ethanol mixture in order to receive a crude saponin extract which was further purified by solid phase extraction.

Separation of the quinoa saponins was performed by HPLC on an analytical reversed phase column using a water-acetonitrile mixture as mobile phase. Detection was performed by a wavelength detector at 210nm and a mass spectrometer using electrospray positive mode ionisation which received the quasimolecular $[M+Na]^+$ ions of the corresponding saponins.

More than 20 different saponins were detected. These saponins were compared with saponins found in the literature. There are 4 aglycons known such as oleanolic acid, phytolaccagenic acid, hederagenin and 30-*o*-methyl sperulagenate. Different sugars are linked to these aglycons at position 3 and 28, at position 28 there is always glucopyranose. At position 3 combinations of glucopyranose, galactopyranose, arabinopyranose, glucuronopyranose and xylopyranose are linked. The number of sugars linked to the aglycon at position 3 varies from 1 to 3.

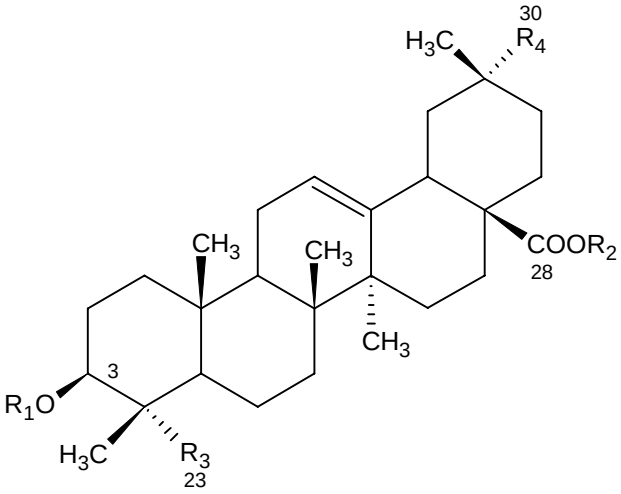

Figure 1. Quinoa Saponins

R_1: combinations of glucopyranose, galactopyranose, arabinopyranose, glucuronopyranose, xylopyranose
R_2: glucopyranose

aglycon	**R_3**	**R_4**
oleanolic acid	CH_3	CH_3
phytolaccagenic acid	CH_2OH	$COOCH_3$
hederagenin	CH_2OH	CH_3
30-*o*-methylsperulagenate	CH_3	$COOCH_3$

One saponin was selected for isolation from the mixture. This was performed by column chromatography of the crude extract using silica as stationary phase and a chloroform-methanol-water mixture as mobile phase. Further purification was performed by HPLC using a preparative reversed phase column and a water-acetonitrile mixture as mobile phase.

Structural analysis was performed by different 1D and 2D NMR experiments such as ^1H, ^{13}C, HMQC, HSQC, HMBC, COSY, TOCSY, NOESY.

P-41

C. Magnes, C. Gatschelhofer, T. R. Pieber, F. M. Sinner

SEPARATION PERFORMANCE OF DIFFERENT STATIONARY PHASES FOR HUMAN INSULIN AND INSULINANALOGA AND QUANTIFICATION LIMITS BY LC MS/MS

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Diabetes mellitus, long considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century. The past two decades have seen an explosive increase in the number of people diagnosed with diabetes worldwide [1].

Insulin therapy is the therapy of choice for type 1 diabetes. During recent years, considerable attention has been devoted to the development of insulin analogues with pharmacokinetic profiles differing from those of existing insulin preparations [2].

Insulin analogues have exchanged or modified amino acid residues. In most cases, there is only a slight difference between the analogues and human insulin. To investigate the pharmacokinetics of insulin analogues in clinical studies, it is neccessary to determine the native hormone and the analogue in one sample. Using immunoassays, seperate assays have to be used for each type of insulin. Analogues often show cross-reactivity to human insulin and the comparison of different assays is difficult. LC/MS is a technique to determin insulins simultanously because it is able to distinguish insulin and insulin analogues by different chromatographic properties and different masses and therefore improve the qualitiy of parallel determinations of insulins.

Herein we report separation performances for insulin and insulin analogues by LC/MS. To achieve the required sensibility for physiological concentrations of insulin we use capillary HPLC-columns coupled with a sensible Triple Quad Mass Spectrometer (Finnigan Quantum Ultra). Three stationary phases – Vydac C4, 300Å, Zorbax SB300-C3, 5µm both 25cm x 300µm and monolithic columns 25cm x 200µm ID produced in our laboratory [3] will be compaired. First results will be reported for the sensibility of insulin quantification using nano-LC-MS/MS (45-1000 amol insulin on column, R^2=0.9998).

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P-42

A. Megyeri, J. Kaplan, J. F. Eliason

LASER SCANNING CYTOMETRY FOR SELECTION OF GREEN FLUORESCENT TRANSGENIC MICE USING SMALL NUMBER OF BLOOD CELLS

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Background: Green fluorescent protein (GFP) transgenic mice can be used for detecting bone marrow microchimerism in transplantation and maternal/progeny models. In order to establish a rapid assay for GFP expressing cells, we have used Laser Scanning Cytometry for quantitative measurements of fluorescence intensity in cells deposited on slides. The position of each detected cell is stored by the Laser Scanning Cytometer (LSC, CompuCyte Corp, Cambridge, MA, USA) allowing the re-location and re-investigation of cells.

Aim: The primary purpose of this study was to identify the GFP+ progeny of a GFP+ female and GFP- male mouse cross to estimate the sensitivity of the LSC method for detection of small numbers of GFP positive cells among the GFP negative cells.

Materials and methods: Adult female C57BL/6-TgN(ACTbEGFP)1Osb mice hemizygous for "enhanced" GFP (EGFP) cDNA under the control of a chicken beta-actin promoter and cytomegalovirus enhancer were mated with adult male wild-type C57BL/6J mice. The test progeny were bled (<0.1ml/mouse) from the retroorbital plexus at 21-35 days after birth. Positive and negative control blood was similarly obtained from adult GFP-hemizygous (GFP+/-) mice and wild type C57BL6/J (GFP-/-) mice, respectively. After dilution with 1 ml of normal saline containing 10 units/ml heparin, the mononuclear cells were separated with density gradient centrifugation. The separated cells were fixed in 1% paraformaldehyde, deposited on slides using a cytospin centrifuge and a coverslip was placed on top with 50% glycerol. The forward scatter of the Ar-ion laser was used for the detection of the cells and the fluorescence was detected in the green channel of the LSC. For the sensitivity estimation, GFP+/- and GFP-/- cells were mixed at 1:1000 ratio and were detected based on their green fluorescence. The detection limit was determined using a negative control sample.

Results: The GFP-/- and GFP+/- mice were separated well based on the measured green fluorescence intensities in the mononuclear blood cells. With an arbitrarily chosen, but fixed level of fluorescence intensity, only 1-5% of cells from GFP-/- mice were above this level, while for the GFP+ mice 80-95% of cells were above the same level. The ratio of the mean fluorescence intensities in GFP-/GFP+ mice was between from 1:8 to 1:14. In mixed GFP+ and GFP- cells (at 1:1000 ratio), using a fluorescence intensity limit which ensured high specificity, we were able to detect 5-15% of the theoretically detectable GFP+ cells.

Conclusion: This method proved to be reliable and fast for the identification of the GFP+/- progenies of GFP+/- x GFP-/- parents, using <0.1 ml of blood. However, because of the relatively high autofluorescence of GFP- cells in the green channel and because of the high heterogeneity of green fluorescence among GFP+ blood cells, the sensitivity of detecting the GFP+ cells in a GFP- population with high specificity is estimated approximately $1:10^4$.

Source of funding: This work has been supported by an American Cancer Society UICC International Fellowship for Beginning Investigators (Attila Megyeri).

P-43

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THE CAROTINOID COMPOSITION IN THE FRUIT OF GOLDEN DELICIOUS APPLES

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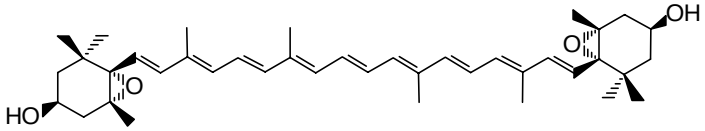
Consumption of fruits and vegetables has been shown to be effective in the prevention of chronic and degenerative diseases, including cancer [1]. Fruits and vegetables contain many compounds, including phenolic compounds, thiols, carotenoids, tocopherols, and glucosinolates that may exert a chemopreventive effect through a variety of mechanisms [2]. The high content of phenolic compounds and carotenoids, the antioxidant and antiproliferative activity of apple peels indicate that they may impart health benefits when consumed and should be regarded as a valuable source of antioxidants [3]. Most previous studies were related to β-carotene, but in recent years, additional carotenoids found in the human diet have also begun to receive attention [4]. In this study our research focuses primarily on the carotenoids of *Golden Delicious* apples.

The HPLC analysis of the total extract (MeOH/Et_2O) of *Golden Delicious* apple (flesh + peel) after saponification (30% KOH/MeOH) in heterogeneous phase resulted in the following carotenoids in the order of their decreasing polarity: an unidentified carotenoid (2.2%), (all-*E*)-neoxanthin (9.3%), (9*Z*)-neoxanthin (2.9%), (all-*E*)-violaxanthin (20.0%), luteoxanthin (4.0%), (9*Z*)-violaxanthin = violeoxanthin (27.3%), antheraxanthin (2.4%), epimers of mutatoxanthin (1.7%), lutein (4.2%), (9*Z*)- and (9'*Z*)-lutein (3.2%), (13*Z*)-, (13'*Z*)- and (15*Z*)-lutein (2.7%), an unidentified carotenoid (1.6%), β-cryptoxanthin-5,6-epoxide (2.3%), (*Z*)-isomer of β-cryptoxanthin-5,6-epoxide (1.2%), β-cryptoxanthin (3.2%), (*Z*)-isomer of β-cryptoxanthin (~0.8%), β-carotene (3.1%), (*Z*)-isomer of β-carotene (0.6%). The identification of the carotenoids was based on their UV/VIS spectroscopic properties and co-chromatography with authentic samples. In addition, the HPLC analysis of the total extract of the peel and the HPLC- and CC analysis of hypophasic fractions of the above mentioned extracts have also been carried out.

The main carotenoids of the hypophasic fractions ((all-*E*)-neoxanthin, (all-*E*)-violaxanthin, (9*Z*)-violaxanthin and lutein) were isolated in pure crystalline state and were characterized by spectroscopic methods (UV/VIS, ^{1}H-NMR, CD, MS).



Violaxanthin

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P-44

C. Moser, I. Klimant

SELF ASSEMBLED SEDIMENTATION ARRAYS BASED ON FLUORESCENCE ENCODED MICROSPHERES

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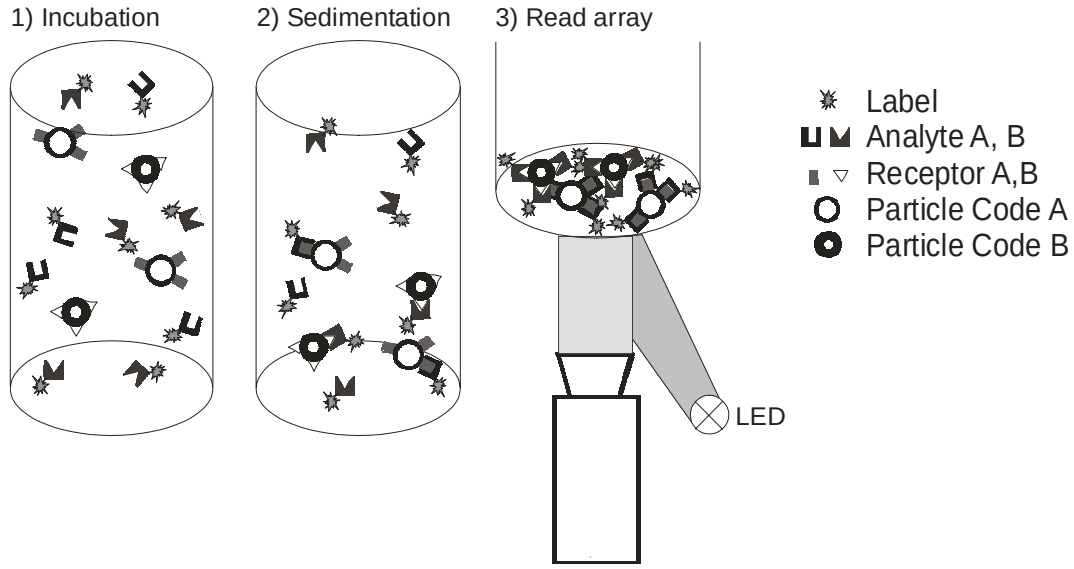
Sensor arrays provide an architecture for parallel multianalyte sensing. On conventional microarrays (immuno- or DNA-chips) each individual receptor is addressed by its defined position on the slide. Another way to address a receptor is to bind it onto the surface of micro spheres carrying a defined fluorescent code. A number of such individual micro spheres (i.e. sensors) can be randomly attached to the distal end of an imaging glass fiber bundle forming a randomly ordered sensor array[1]. It is also possible to disperse these particles in a solution and to analyze them in a flow cytometer[2].

Here we introduce a new concept towards multiplexed analysis including advantageous features of both approaches mentioned above. The use of fluorescence-encoded micro spheres of significantly higher density than water results in sedimentation in the range of minutes. During this sedimentation the binding of the analyte onto the receptor modified particle surface occurs. This leads to *in situ* formation of randomly ordered sensor arrays e.g. at the transparent bottom of each individual well of a microplate. This *in-situ* formed array is then evaluated by an inverted fluorescence microscope equipped with a CCD-camera that is connected to an automated image analysis system. The concept has the potential of fast and flexible creation of user-defined arrays.

The selection of appropriate particles based on different materials, different types of encoding schemes and the image analysis are discussed. The proof of principle is demonstrated. A critical comparison with other established concepts for sensor arrays is given.

Schematic drawing of self assembled sedimentation array principle



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P-45

L. Nagy, G. Nagy, L. Kollár

ELECTROCHEMICAL BEHAVIOUR OF SOME ELECTROACTIVE ORGANIC MOLECULES IN IONIC LIQUID MEDIA

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In the early period of electrochemistry experiments with molten salts were common and they resulted in major discoveries. In our time electrolysis of molten electrolytes is used in industrial scale. As it is well-known, molten ionic crystals, e.g. molten cryolite, can be used as good solvents providing optimal conditions for the electrode reactions.

On the other hand, electrochemical experiments in molten ionic compounds are not often carried out in research laboratories. The reason is obvious. The high temperature work needs special cells, electrodes, apparatus, conditions and special care. Furthermore most of organic electroactive compounds would decompose at high temperature.

Chemistry and applications of ionic liquids, organic salts with melting point below or just slightly above ambient temperature started to draw considerable interest in the last few years [1]. Their use in other areas of electrochemistry, or in electro-analytical chemistry seems also very promising. Extended potential window, lower cell resistance, decreased electrode passivation, beneficial influence on different steps of electrode processes can be advantages gained by their application.

Most recently we started investigating the voltammetric behavior of different electroactive species in 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIM^+$ PF_6^-) ionic liquid at room temperature. In these studies small volume measurement cell, conventional size and ultra micro platinum and carbon (Glassy Carbon and carbon fiber) working electrodes were used. Cyclic voltammetric, and chronoamperometric measurements were performed.

An advantageously broad potential window for the voltammetric measurements was found in $BMIM^+$ PF_6^-. Ferrocene, p-nitro-phenol, Meldola blue and methylene blue were used as electroactive model compounds. The ferrocene and the Meldola blue showed quasi reversible voltammetric character. The i_p- $v^{1/2}$ dependences for all species were linear indicating diffusion character of the voltammetric peaks. Linear concentration dependence of the oxidation and reduction peaks were found in cases of the species studied.

The diffusion coefficients of the different species were estimated from the slopes of i_t/i_∞ - $t^{-1/2}$ plots of the chronoamperometric curves measured with ultra microelectrodes, and the number of electrons exchanged in the electrode processes was checked.

Interestingly the electrodes fouled by passivating film formed in the electrochemical oxidation of p-nitro-phenol could be regenerated at -1.8 – -2.0V polarizing potential in $BMIM^+$ PF_6^- ionic liquid.

The details of experimental techniques and the results obtained will be demonstrated in the poster.

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P-46

L. Nagy, R. Bátai, G. Nagy, P. Hajós

COPPER ELECTRODE BASED AMPEROMETRIC DETECTION OF SUGARS AND ORGANIC ACIDS FOLLOWING ION CHROMATOGRAPHIC SEPARATION

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The amperomertic detection proved very advantages in flow analysis, especially if a previous separation step, such as HPLC, IC, or CE is involved in the procedure.

Using it, very low detection limit can be achieved measuring electroactive species. Most often conventional precious metal or carbon based voltammetric electrodes with broad potential windows and low residual current are used as sensing element in the detector cells. If simple constant potential amperometry fails because of electrode fouling or other complications, pulsed amperometric technique can be the solution. In some cases, however sensing electrodes with narrow potential windows, seldom used in potential scanning methods, have been advantageously used in constant potential amperometry. Among them electrodes made of Ni, Cu, or different electrodes holding immobilized electrocatalytic units can be mentioned.

In our work the applicability of copper based sensing electrodes were investigated in constant potential amperometric measurement of different monosacharides and carboxylic acids. A thin layer type flow cell incorporating a homemade copper sensing electrode was fabricated. Well-defined dependence was found between the analyte concentration and the constant potential amperometric response in case of different analytes in basic solution. The detector cell was used in flow injection analysis (FIA), ion-chromatography (IC) and in high performance liquid chromatographic (HPLC) measurements. The nature of the electrode process responsible for the amperometric response was investigated in case of the different analytes.

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P-47

A. Nagy, B. Bugyi, A. Málnási-Csizmadia, A. Lukács, D. Lőrinczy

STRUCTURAL STABILITY OF CHEMICALLY DENATURED GREEN FLUORESCENT PROTEIN

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Green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* is a 28 kDa (238-aa residues) barrel-shaped molecule, 24 Å in diameter and 42 Å in length. Outer "layer" of the barrel is composed of 11 antiparalell β-sheets, which connected with α-helical stretches. One α-helix extends to the interior of the "β-can" and forms the fluorescent chromophore. Chromophore is composed from three (-Ser^{65}-Tyr^{66}-Gly^{67}-) postranslationally-modified amino acids. GFP converts the blue light into a brilliant green fluorescence (λ_{max} = 508-509 nm).

The aim of our work was the determination of structural changes in GFP in presence of denaturating agent (GuHCl). Here we report on the thermal stability and changes in fluorescence in presence of 0 M - 6 M of GuHCl.

Thermal stability of GFP can be monitored with differential scanning calorimetry. Our results indicate, that in 1-6 M range of denaturating agent the melting temperature is decreasing continuously from 83 to 38 ºC. The calculated calorimetric enthalpy decreases with GuHCl concentration up to 3 M (56 → 2 J/kg), but at 4M it jumps to 84 and at greater concentration it is falling back to 11 J/kg. First phenomena, i.e. the decrease of melting point with increasing GuHCl concentration can be easily explained by the effect of the extended chemical denaturation, when less and less amount of heat required to break the remaining hydrogen bonds in β-barrel. The surprising increase of calorimetric enthalpy at 4M concentration of GuHCl could be the consequence of a dimerization or a formation of stable complex between GFP and denaturing agent.

Similarly to these results, in range 0 - 3 M of GuHCl the intensity changes in exiguous manner. When we applied GuHCl in concentration of 4 to 6 M, the intensity of steady-state fluorescence decreased to zero.

From our results we conclude, that in the 0-3 M GuHCl range GFP gradually looses its compactness and becomes more flexible, but the structure remains intact. From 4 to 6 M GuHCl the hydrogen bonds between β-sheets become completely broken and in result the GFP unfolds.

P-48

J. Németh, B. Jakab, M. Thán, Zs. Helyes, Á. Szabó, E. Pintér, J. Szolcsányi

RADIOIMMUNOASSAY MEASUREMENT OF SENSORY NEUROPEPTIDE RELEASE INDUCED BY CAPSAICIN RECEPTOR (VR1/TRPV1) AGONISTS AND ELECTRICAL FIELD STIMULATION *in vitro*

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Neuropeptides like substance P and calcitonin gene related peptide (CGRP) released from the peripheral terminals of capsaicin-sensitive, VR1/TRPV1 receptor-expressing primary sensory neurones in response to orthodromic (chemical) or antidromic (electrical) stimulation induce local neurogenic inflammation in the innervated area. Furthermore, activation of these fibres exerts systemic anti-inflammatory activity mediated by the released somatostatin. In the present study the release of these three peptide mediators from isolated rat tracheae in response to different concentrations of VR1 agonists like capsaicin, resiniferatoxin (RTX) and piperine or electrical field stimulation with various number of pulses was determined with radioimmunoassay (RIA).

Release study: Tracheae of two adult Wistar rats were perfused with oxygenated and pH-controlled (pH: 7.2) Krebs-solution with flow rate of 1 ml/min in an organ bath (1.8 ml) at 37 °C for 60 min. After equlibration, the flow was stopped and three 8-min fractions (prestimulated – stimulated – poststimulated) were collected. Electrical field stimulation (40 V, 0.1 ms, 30 - 1200 pulses) or VR1 agonists (capsaicin: 10^{-8} – 10^{-5}, RTX: 10^{-10} – 10^{-7}, piperine: 10^{-6} – $5x10^{-5}$ mol/l) were applied in the second 8-min period (stimulated fraction) to induce neuropeptide release. Substance P, CGRP and somatostatin contents of the collected incubation medium were measured and expressed as the released amount of peptides per wet tissue weight (fmol/ mg).

Results and discussion: Electrical field stimulation at frequency ranging from 0.5 to 10 Hz is effective to release substance P, CGRP and somatostatin from sensory nerve endings of rat tracheae, and the amount of peptide outflow is dependent on the number of pulses. It has also been shown that capsaicin, RTX or piperine evoke a dose-dependent release of these sensory neuropeptides and all three neuropeptides are released by the three examined VR1 agonists in similar extent. Their potency, however, differ significantly. In respect of sensory neuropeptide releasing potency, RTX is approximately 80-100 times more potent than capsaicin, but it requires longer time to reach its maximum effect. Capsaicin is approximately 50-70 times more potent than piperine.

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P-49

E. Panciroli, U. Zeipper, P. Sedlmayr, R. Wintersteiger

QUANTIFICATION OF KYNURENINE IN CERVICAL MUCUS AS INDICATOR FOR INDOLEAMINE 2,3-DIOXYGENASE ACTIVITY

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Institute of Histology and Embriology, University of Graz

Kynurenine is a metabolic product of tryptophan metabolism produced by indolamine 2,3-dioxygenase (IDO). This enzyme appears to play an important role in regulation of feto-maternal tolerance and protection against ascending infections in the female reproductive tract. Therefore, an HPLC method has been developed in order to determine kynurenine in small quantities of cervical mucus and thus making possible calculation of IDO activity.

After preincubation of the cervix sample a standard reaction mixture consisting of methylene blue, ascorbic acid, catalase, L-tryptophan, phospate buffer, aprotinine and leupeptin was added. This composition of agents is needed to protect against oxidation and proteolysis. Following incubation for 30 min. the reaction is terminated by adding trichloroacetic acid and further incubated to hydrolyse N-formylkynurenine to kynurenine. To preclean the complex mixture containing numerous interfering by-products in a highly viscous solution a solid-phase extraction system with C18 cartridges has been developed as well. Using 40% methanol and acetonitrile acceptable recoveries could be obtained. HPLC separation is performed applying a C18 reversed phase system. Changing the wavelength from 360 nm for the kynurenine analysis to 274 nm for the tryptophan analysis the relationship of these both compounds can be determined within one run. Also kynurenine without reaction mixture has to be analysed for exact calculation of IDO activity. The method developed shows good reproducibility and allows IDO activity determination via kynurenine quantification in the picomole range/h/g cervical mucus.

P-50

Á. Pénzes, K. Majdik, F. Kilár, F. dan Irimie

BIOSORPTION OF CR(VI) AND FE(III) IONS BY BAKER'S YEAST „*SACCHAROMYCES CEREVISIAE*"

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Cr(VI) and Fe(III) are common and toxic pollutants in soils and wastewaters. Results of earlier studies on the metal bioaccumulation processes can also be applied in biotechnology, i. e. yeasts can be used for the biosorption of heavy metals from the environment. Microorganisms remove heavy metal ions by a number of different processes.In order to clarify the heavy metals-microorganisms interactions we used Saccaromyces cerevisiae and Cr(VI), Fe(III) stock solution.We could see that the first stage of the biosorption is rapid and occurs a short time after the yeast have come into contact with the metal.After this time the biosorption is slower.The temperature influenced the quantity of the biosorped heavy metals.In some study we used immobilized yeast to perclude the biosorption on the cell walls.The results showed that the biosorption on the cell wall play a very important step in the heavy metals remove.The changes of the yeast fermentation and the effect to the biosorption of heavy metals will be studied in further investigation.

P-51

G. Petőcz, Z. Berente and L. Kollár

PENTACOORDINATED PLATINUM(II) COMPLEXES: AN NMR STUDY

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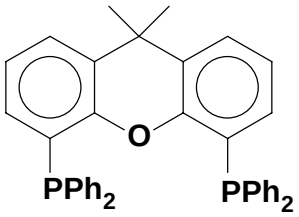
It has been known for a long time that both steric and electronic parameters of the ligands have impact on their coodination properties [1]. Recently, emphasis has been put on the synthesis of ligands with specific geometries. It has been found that bidentate ligands can have a preference for a specific geometry, since the *bite angle*, defined as donor atom(1)-metal-donor atom(2) angle, is dependent on the bridge between the two donor atoms. The majority of the ligands applicated up to now prefer the donor atom-metal-donor atom bite angle close to 90^o (typically between 85 and 95^o), so tetragonal rather than tetrahedral geometry is favoured from sterical reasons. However, recently van Leeuwen *et al.* have shown that the *xantphos* ligand family, enforcing bite angles between 100-120^o, tends to occupy the bisequatorial position rather than an axial-equatorial position in trigonal pyramidal Rh(I) complexes [2].

In this paper we describe the ^{31}P NMR characterisation of platinum complexes containing "PP_3" ($P(CH_2CH_2PPh_2)_3$, **1**) and *xantphos* (**2**) ligands. Depending on the above-mentioned structural factors, both the formation of the well-known tetragonal platinum(II) complexes and that of the pentacordinated species with trigonal bipyramidal (*tbp*) geometry could have been observed. It is shown that steric factors and the bite angle of the ligand could result in unique structures which shed light to important steps of homogeneous carbonylation, *i.e*. they might explain the existence of some key intermediates in the catalytic cycle.



xantphos

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P-52

A. Petz, Z. Pintér, L. Kollár

MASS SPECTROMETRIC STUDIES ON THE COMPLEX REACTION MIXTURES OBTAINED UNDER CARBONYLATIVE 'SUZUKI-CONDITION'

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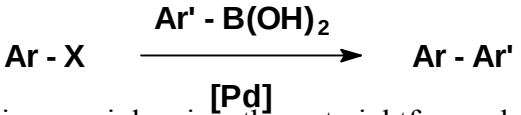
Among C-C bond forming reactions the palladium catalysed cross-coupling of organoboronic acids or organoboronates with organic electrophiles (referred usually as Suzuki reaction [1]) turned out to be a versatile reaction as shown by several reviews [2-4].

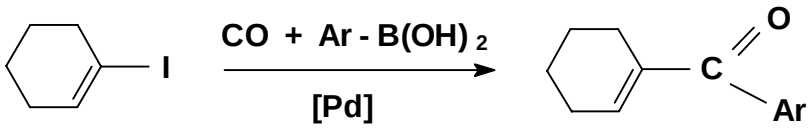


Scheme 1

Its importance lies mainly in the straightforward synthesis of oligoaryls (oligoheteroaryls) or dienes in aromatic or vinylic couplings, respectively, under mild reaction conditions. The Suzuki reaction has gained a special importance in the last few years because the conditions developed for this reaction have many desirable features for large-scale synthesis of pharmaceuticals and fine chemicals. The boronic acids and esters proved to be more user-friendly and environmentally safer than the other main-group organometallic reagents, such as the Grignard reagents and organostannanes. A review focused also on these topics has been published quite recently [5].

The application of aryl halides or triflates predominates in Suzuki reaction and only sporadic results can be found for alkenyl halides or triflates.

The aim of this work was to synthesise unsaturated ketones under carbonylative 'Suzuki conditions'. The 1-iodo-1-cyclohexene model substrate underwent not only 'normal' (*Scheme 1*) and 'carbonylative' (*Scheme 2*) coupling, but side-reactions like Heck-reaction of the product(s) with the unreacted substrate have also observed. GC-MS proved to be a powerful tool for the routine analysis of rather complex catalytic mixtures.



Scheme 2

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P-53

A. M. Piccinini, M. G. Schmid and G. Gübitz

CHIRAL SEPARATION OF NATURAL AND NON-NATURAL AMINO ACID DERIVATIVES BY MICRO-HPLC ON A RISTOCETIN STATIONARY PHASE

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Amino acids represent important components in nature and are building blocks of proteins. Although the L-enantiomers are predominant, D-amino acids have been found in nature and have been recently shown to play a certain role in human physiology. Therefore, the development of analytical methods for the chiral separation of amino acids and the identification of the enantiomers attracted increasing interest.

This work deals with the chiral separation of derivatives of natural and non-natural amino acids by micro-HPLC. The non-biogenic amino acids contain a sulfonamide moiety, which are used as building blocks for peptide synthesis. As a stationary phase, ristocetin A, chemically bonded to 3.5 µm silica gel was used. This material was packed into 10 to 20 cm columns of 1 mm ID. Ristocetin A, a macrocyclic glycopeptide antibiotic, derives from the fermentation of *Nocardia Lurida* and consists of an aglycon portion of four macrocyclic peptide rings and several sugars. Its 38 stereogenic centers, 7 aromatic rings, 5 6 amide linkages, 21 hydroxyl groups, 2 amino groups and one methyl ester allow chiral recognition through hydrophobic interactions, hydrogen bondings, dipole-dipole interactions and π–π interactions.

The equipment consisted of a normal HPLC pump with a passive split and a conventional UV-detector with a micro flow-cell. The advantages of such a miniaturized system are less solvent-, chiral stationary phase- and sample consumptions. A broad spectrum of compounds was resolved with this system, whereby normal phase-, polar organic- and reversed phase mode were compared.

P-54

P. Raimann M.R. Wagner, K-C. Möller, J.O. Besenhard, M. Winter

ELECTROCHEMICAL DILATOMETRY AND MASS SPECTROMETRY STUDIES OF LITHIUM ION BATTERY ANODES

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Differential Electrochemical Mass Spectrometry (DEMS) is known as a useful tool for the in situ measurement of volatile species that are evolved at an electrode of an electrochemical cell. The method is based on a porous hydrophobic membrane that works as a solvent barrier between the electrochemical cell and the vacuum system of the mass spectrometer. Gases that evolve at the working electrode are sucked through the membrane and analysed in the mass spectrometer [1].

A technique known as in situ electrochemical dilatometry can be used to record the macroscopic expansion (dilatation) and contraction of lithium ion battery electrodes during charge/discharge in Li+ cation-containing nonaqueous electrolytes. Such dilatations and contractions are found in crystals having layered lattice when guest ions undergo intercalation/deintercalation reactions. These changes of crystal dimensions (thickness) can be monitored in situ, simultaneously with electrochemical parameters such as the electrode potential, when the dilatometry is performed in combination with some electrochemical technique (cyclic voltammetry) [2].

This contribution will present dilatometric and DEMS investigations on carbon and metal based anode materials for lithium ion batteries.

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Support by the Austrian Science Funds through the special research program "Electroactive Materials" is gratefully acknowledged

P-55

R. Raml, W. Goessler, K. A. Francesconi

μ-HPLC-ICPMS FOR DETERMINING ARSENIC SPECIES IN HUMAN URINE

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Arsenic is ubiquitous in small amounts in the environment. It is present in many different chemical forms [1] that differ significantly in their toxicity. The major sources of arsenic in our food chain are drinking water (containing mainly toxic inorganic arsenic species) and seafood (containing non-toxic arsenobetaine as the major arsenical). Thus, in order to assess possible health risks from ingestion of arsenic, the chemical form of the arsenic must be determined.

A convenient method to determine different arsenic species at low concentrations is to couple HPLC to an ICPMS [2], and this technique (HPLC-ICPMS) is the most common way of performing arsenic speciation analyses. When dealing with biological samples, however, the small sample volumes (often only a few microlitres) restrict the use of conventional HPLC, and have lead to the development of μ-HPLC systems. Although the advantages of μ-HPLC, like the ability to handle small sample amounts, good resolving power, and easy coupling with mass spectrometers are already widely exploited in bioanalysis and protein/peptide research [3], there have been few reports of the use of μ-HPLC for arsenic speciation analyses.

In our work methods were developed that allow the separation of five cationic arsenicals (arsenobetaine, the glyceryl-arsenoriboside, trimethylarsine oxide, arsenocholine, and the tetramethylarsonium cation) and four anionic arsenicals (arsenite, arsenate, methylarsonate, and dimethylarsinite employing μ-HPLC coupled to an ICPMS, which served as the arsenic specific detector. Detection limits and analysis times achieved with μ-HPLC were comparable to those obtained with conventional HPLC. The method was used to determine natural occurring arsenic species in human urine. First arsenobetaine in a urine standard reference material was determined and the measured concentration (65 ± 1 μg L^{-1}) was found to be in good agreement with the certified value (69 ± 12 μg L^{-1}). Other metabolites like arsenite, arsenate, methylarsonate, and dimethylarsinite were determined in a spiked urine sample. The results were found to be in good agreement with those from measurements with a different method (HPLC-HG-ICPMS).

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P-56

G. Rangits, Gy. Petőcz, Z. Berente, L. Kollár

THE NMR INVESTIGATION OF Pt-DIPHOSPHINE-TIN(II)CHLORIDE SYSTEMS IN [BMIM][PF_6] IONIC LIQUID

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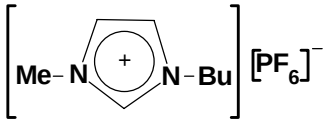
Department of Biochemistry and Medical Chemistry, University of Pécs, Medical School, H-7624 Pécs, Szigeti út 12, Hungary

The application of ionic liquids as 'alternative solvents' in homogeneous catalytic reactions turned up in the middle of the nineties, and reviewed recently [1-3]. Although several types of catalytic reactions have been studied, the coordination chemistry and elementary reactions of complexes concerning the mechanistic details have scarcely been investigated.

The formation of platinum-diphosphine complexes of catalytic importance was investigated in [BMIM][PF_6] (BMIM = butyl-methyl-imidazolium cation) ionic liquid by NMR for the first time.



Both the $PtCl(SnCl_3)(bdpp)$ (**1a**) and $Pt(SnCl_3)_2(bdpp)$ (**1b**) complexes (where *bdpp* = 2,4-bis(diphenylphosphino)pentane) were identified in the insertion reaction of tin(II)chloride into the Pt-Cl bond of $PtCl_2(bdpp)$ complex. The addition of PPh_3 to $PtCl_2(bdpp)$ resulted in the formation of $[Pt(bdpp)(PPh_3)Cl]^+$ complex cation (**1c**) as a minor product, which turned to be a major product in the presence of tin(II)chloride while it forms trichlorostannate counterion.

$$\underset{1}{PtCl_2(bdpp)} + SnCl_2 \rightarrow \underset{1a}{PtCl(SnCl_3)(bdpp)} + SnCl_2 \rightarrow \underset{1b}{Pt(SnCl_3)_2(bdpp)}$$

$$\underset{1}{PtCl_2(bdpp)} + PPh_3 + SnCl_2 \rightarrow \underset{1c}{[Pt(bdpp)(PPh_3)Cl][SnCl_3]}$$

The addition of a diphosphine different from that in $PtCl_2$(diphosphine) complex resulted 'PtP_3' species in typical AMX second-order spectra with $^2J(P,P)$ and $^1J(Pt,P)$ of diagnostic value in ^{31}P NMR.

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P-57

M. Reischl , A. Bizzarri, W. Trettnak , G. Uray , V. Ribitsch

OPTOCHEMICAL CARBON DIOXIDE SENSOR BASED ON AN EUROPIUM(III) CHELATE AND LUMINESCENCE LIFETIME MEASUREMENT

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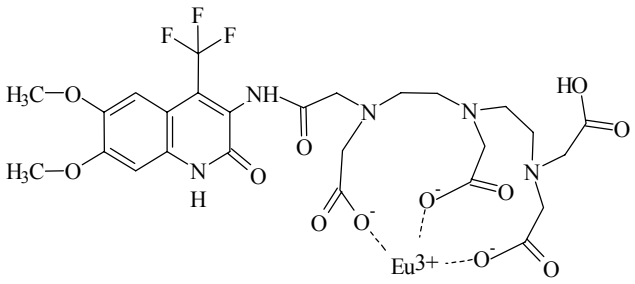
Luminescent lanthanide chelates, especially of Eu^{3+} and Tb^{3+}, have found multiple applications such as luminescent probes in immunoassays and in resonance energy transfer experiments and as contrast agents in magnetic resonance imaging. Beside the mainly medical and biochemical applications, there is a growing interest in utilising these chelates for opto-chemical sensing due to their uncommon spectral properties. The spectral characteristics include large Stokes shifts (>150 nm), spiked emissions and luminescence decay times up to the millisecond range.

A 4-trifluoromethylcarbostyril derivative of diethylenetriamine-pentaacetic acid (DTPA) forms a stable chelate with Eu^{3+}-ions (see Figure!), whereby the carbostyril serves as antenna molecule, with an excitation maximum at 370 nm. The complex shows typical antenna-mediated lanthanide luminescence with the most intense peak at 615 nm [1].



Furthermore, a strong pH dependence of both luminescence intensity and lifetime is observed [2]. The chelate is a pH indicator with an apparent pKa of approximately 8.2 in aqueous solution, displaying decay time changes from 520 µs to 35 µs in the range from pH 7 to 9.

The presented novel pCO_2 sensor is able to overcome several problems occurring in previously reported sensing methods. The chelate combines pH sensitivity and long luminescence decay times, so there is no need for an additional pH indicator as with energy-transfer-based sensor systems. On the other hand, the dye is not quenched by oxygen, a well-known problem common to all ruthenium complex based pH and CO_2 sensors. The excitation maximum at 370nm enables the application of new UV-LEDs as light source. Considering the long luminescence decay times, time resolved sensing at low LED modulation frequencies is facilitated, thus eliminating the drawbacks of intensity measurements.

The CO_2 sensor is based on a previously reported opto-electronic setup utilising a phase measurement technique [3] and a measurement frequency of 183 Hz. Various amounts (4-16 µl) of a dye/buffer solution were entrapped by means of a gas-permeable Teflon membrane (thickness ca. 10 µm) in a specially designed exchangeable sensor cap. Concentration and type of the buffer solution are the main factors controlling the dynamic range and sensitivity of the sensor and were adjusted to a pCO_2 measurement range of 10-150 hPa. Typical response times (t_{90}) of the sensor were found to be in the order of 10 min.

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P-58

Th. Riedl. C. Schür, T. Marek, H.P. Strunk, S. Kunsági-Máté

RAMAN ANALYSIS OF THE DEFECT POPULATION IN GaAs GROWN AT LOW TEMPERATURE

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Raman spectroscopy provides a powerful tool to identify different types of defects in GaAs layers grown at low temperature of about 200°C – 400°C (LT-GaAs) by Molecular Beam Epitaxy (MBE). These layers are As-rich, crystalline and reveal high concentrations of atomic and complex defects. They are of interest for e.g. high-frequency opto-electronic applications because of their ultra-short lifetime of photogenerated carriers (<1 ps).

In detail, Raman spectra contain information about the content of As_{Ga} antisites, As interstitials As_i and As nanoclusters. With increasing As_{Ga} antisite concentration one observes a shift of the LO phonon frequency to lower values. In the examined LT-GaAs layers we determined shifts of about 1.5 cm^{-1} with respect to the 600°C standard GaAs layers, corresponding to a relative As_{Ga} antisite concentration of about 3.3%.

Furthermore, As_i interstitials and As clusters give rise to characteristic features in the Raman spectra. While a broad peak at 222 cm^{-1} is caused by As_i interstitials, As nanoclusters are accounted for peaks at 200 cm^{-1} and 255 cm^{-1}. The intensity of these peaks in relation to the LO intensity is a measure for the defect content. LT-GaAs samples grown on substrates with a surface orientation which deviates from the usual (001) off to the <111>A direction show an even higher defect intensity ratio compared to LT-GaAs on exactly (001) oriented substrate.

By changing the excitation laser wavelength we have the possibility to estimate the defect concentrations as a function of the depth below the layer surface. According to an absorption length of around 100 nm, blue light ($\lambda \approx 488$ nm) probes regions near the surface; red light with $\lambda \approx 633$ nm provides information of a ≈ 300 nm deep volume. Using these wavelengths we found the As_i interstitial content to be slightly higher in the bulk than near the surface.

From these results we can draw conclusions on the MBE growth process of GaAs. We applied respective calculations of the defect formation, particularly on the misoriented surface. These calculations suggest that the higher As_i interstitial content in <111>A misoriented samples is due to favoured incorporation of As_i interstitials at misorientation steps parallel to the [1,-1,0] direction on the (001) surface.

P-59

B. Schäffer, S. Szakály, D. Lőrinczy

EXAMINATION OF THE STRUCTURE OF PROBIOTIC CHEESE SPREADS BY ELECTRON MICROSCOPE AND DSC METHODS

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

In most of the world cheese spreads are produced by heat treatment during which processing salt is added. This general technology results on the one hand in the destruction of useful lactic acid bacteria in the cheese or other added dairy products (e.g., sour cream), at the same time that the added phosphate of the processing salt diminishes the otherwise highly favorable Ca:P ratio of the dairy products. The Hungarian Dairy Research Institute (HDRI) has elaborated a production procedure for cheese spread in which the Ca:P ratio is in accordance with the physiological optimum (1:1), and the product contains live lactic acid bacteria in the order of 10^7. A further advantage of cheese spread produced by this procedure is that it remains non-sticking and easily spreadable at both refrigerator (0-7°C) and room temperature (20-24°C), which property very likely has to do with its structure.

For the examination of its structure we used electron microscope and DSC methods. The products examined were traditional processed cheese spread and probiotic cheese spread produced by HDRI technology. For direct observation of the microstructure the samples were fixed, dehydrated and imbedded, then semi-thin slides were checked by optical microscope, and slides of 50 nm thick were made from the blocks which were judged to be best. After staining, the examinations were conducted by JEOL 1200 EX electron microscope in transmission mode. Heat-induced changes in the samples were examined by SETARAM Micro DSC-II ultrasensitive scanning calorimeter. For the examinations the traditional 1 cm^3 batch vessels were used with 700-900 mg of sample masses. Measurements were taken over the range of 0-100°C at a heating rate of 0.3K/min.

From the results of the electron microscope and DSC assays it was determined that the outstanding non-sticking spreadability of the probiotic cheese spreads was in correlation with the more homogenous fat and protein structure. With regard to fat melting no difference was observed between traditional processed cheese spreads and the probiotic cheese spread produced by HDRI technology.

P-60

B. Schäffer, S. Szakály, D. Lőrinczy

ELECTRON MICROSCOPE STRUCTURE OF PROBIOTIC SPREADS, INDICATION OF THEIR PROBIOTIC MICROBES BY ISOPERIBOLIC BATCH CALORIMETRY, AND THEIR HUMAN PHYSIOLOGICAL EFFECT

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Department of Biophysics, University Pécs, Faculty of Medicine.
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Spreads with a fat content of 30-50% (these are called „butter creams" in Hungarian and currently outsell butter) are generally made by post-heat treatment, and consequently contain no probiotic lactic acid bacteria.. The Hungarian Dairy Research Institute (HDRI) has developed a spread which contains live lactic acid bacteria in the order of 10^7/g, some of which are probiotic. The major advantage of the probiotic spread compared to the post-heat treated is that it spreads easily and without sticking both at cold (0-5°C) and at room temperature (20-24°C). This spreadability comes from its structure, while its probiotic quality derives from the proliferation of probiotic microbes. During our experiments the extent of probiotic microbe proliferation was examined by isoperibolic batch calorimetry, the microstructure was studied by electron microscope, and nutritional biology characteristics were measured by human clinical studies.

In order to indicate the probiotic microbes 5% probiotic butter spread was suspended in non-fat sterile milk and the suspension was fermented at 30°C (the fermentation temperature of probiotic spreads) until pH 4.7 was reached: in other words, a butter spread ferment was produced. For the calorimetric measurements cc. 450 mg sterile non-fat milk and approx. 50 mg butter spread ferment were separately put into a mixing batch vessel and left at 30°C until thermal equilibrium was reached. Next the butter spread ferment was injected into the milk, and microbe proliferation was recorded for 12 hours by a SETARAM Micro DSC-II calorimeter at 30°C under isotherm conditions. Total plate titer was determined as described above, but using a thousand times greater quantities. For examination of the microstructure the samples were fixed, dehydrated and imbedded, then slides of 50 nm were made. After staining, the examinations were performed by JEOL 1200 EX electron microscope in transmission mode. In the human clinical examinations margarine and probiotic butter spread were compared by the self-controlled method, in which total, HDL and LDL cholesterol levels in the blood were measured after the consumption.

By deconvoluting the heat flow-time curves it was determined that the probiotic cell titer was in the order of 10^7/g, far in excess of the internationally accepted cutoff of 10^6/g. Structural research shows that the fat globules in probiotic spread are distributed homogenously, with an average diameter of less than 0.5 μm. The findings from the human clinical examinations showed that the reduction in the blood cholesterol level occurring after consumption did not differ significantly in total cholesterol, but probiotic spread effected a significantly greater reduction in the supposedly harmful LDL cholesterol in the third hour followed the consumption, and a less reduction in the HDL cholesterol level.

P-61

B. Schäffer, S. Szakály, D. Lőrinczy

EXAMINATION OF THE CORRELATION OF BUTTER SPREADABILITY AND FAT STRUCTURE BY DSC

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Since the appearance of margarines and spreads on the market, they have been serious competitors with butter. One of the reasons for this was the false nutritional biology propaganda, but today butterfat has scientifically regained its actual nutritional evaluation. The main disadvantage of butter in comparison to other spreadable tallows is that it does not immediately spread as well when taken out of the refrigerator. One method of obtaining better cold-spreadability is appropriate cream ripening in which a different system known as the corpuscular colloid is created. Our earlier electronparamagnetic resonance (EPR) spectroscopic examinations showed that a corpuscular butter structure could be achieved by cream ripening. In these experiments an answer was sought to the question whether a difference could be shown in the thermal properties of homogenous and corpuscular butter structure, and how that difference correlates to the results of the EPR-spectroscope assays.

The examinations were conducted during the winter, when the problem of butter spreadability is the greatest. The cream was ripened by simple cold ripening in accordance with the method used in our earlier EPR studies, and by heat-step ripening, then butter was produced from these ones. Approx. 800 mg of the butter samples were placed into the conventional batch vessels, then the DSC curves were recorded by SETARAM Micro DSC-II calorimeter over a range of 0-50°C at a scan rate of 0.3K/min.

By deconvoluting the DSC curves it was established that

- butter made from the cream ripened by the heat-step method had three characteristic melting peaks as distinct from the two melting peaks of butter made from cold-ripened cream, and
- the temperature of the second melting peak for butter from heat-step cream was identical to the characteristic melting temperature for fat particles from earlier EPR spectroscope assays.

In sum it can be stated that the DSC method clearly shows both the homogenous and the particle structure characteristic of butter.

P-62

B. Schäffer, S. Szakály, D. Lőrinczy

EXAMINATION OF THE STRUCTURE OF PROCESSED CHEESES MADE WITHOUT PEPTIZATION BY ELECTRON MICROSCOPE AND DSC METHODS

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In the calcium supply of the body and the calcium-phosphorus balance, dairy products play the most important role among all foods. Without them neither the daily calcium intake of 800-1200 mg nor the physiologically optimal Ca:P ratio (1:1.5) can be assured. The spread of osteoporosis throughout the world can be traced primarily to low milk and dairy product consumption. This is especially true for Hungary, where milk and dairy product consumption began to drop dramatically in 1990 and continues to decrease today. The dairy products originally have a highly favorable Ca:P ratio (1.4:2.0). This ratio becomes substantially worse (0.53:0.42) when processed cheeses are made with phosphate-containing processing salts. Consequently, Hungarian Dairy Research Institute (HDRI) has elaborated a production procedure through which unpeptized processed cheese can be produced without phosphate processing salt. The procedure makes it possible to increase the calcium content of the product and to adjust the optional Ca:P ratio. In the course of our experiments we conducted electron microscope and DSC examinations of the structure of processed cheese produced by the new method.

The electron microscope examinations included Trappist cheese and processed cheeses made from it with (Mackó brand) and without (Calci) peptization. Samples of approx. 1 mm^3 were fixed and imbedded, then after staining slides of semi-thin were investigated by optical microscope and thin (approx. 50 nm) slides by electron microscope. The electron microscope examinations were conducted by JEOL 1200 EX electron microscope in transmission mode. Heat-induced changes in the peptized and unpeptized processed cheese samples were examined by SETARAM Micro DSC-II scanning calorimeter. For the examinations the conventional batch vessels were used, with 700-900 mg of sample masses. Measurements were taken over a temperature range of 0-100°C at a heating rate of 0.3K/min.

The microscopic and electron microscopic examinations clearly show that homogeneity increases in the direction of cheese – peptized processed cheese – unpeptized processed cheese. This applies to both the protein and the fat phases. From the DSC curves and their deconvolution analysis the following can be stated:

- The heat-induced changes (the endotherm melting of the butterfat) in butterfat being in the more homogenous fat emulsion created by the producing procedure without peptization, were identical to those occurring in butterfat of processed cheeses produced with peptization.
- The temperature range of gel-sol protein transformation of peptized processed cheeses is much higher (40-90°C) than that of unpeptized processed cheeses (40-70°C).

P-63

Ch. Seger, S. Strum, H. Römpp, P.C. Schmid, E. Haslinger, F. Hadacek

CHARACTERIZATION OF LIPOPHILIC PLANT CONSTITUENTS WITH LC-MS AND GC-MS - *HYPERICUM PERFORATUM* L. SUPERCRITICAL FLUID EXTRACTS AS A CASE STUDY

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The workup of interesting lipophilic secondary plant constituent (e.g. terpenoid compounds, phloroglucinol derivatives of economic value or interesting pharmacological data) if often hampered by the occurrence of large quantities of mixtures of waxy, oily or resin like appearance. Working on the optimization of the supercritical fluid extraction process for hyperforin (from *Hypericum perforatum* L.) we were confronted with a pale yellow matrix resting atop the hyperforin enriched phase in the extractor. Using both LC-DAD-MS and GC-MS techniques, we are able to determine the extract composition both qualitatively and quantitatively. Besides the dominating phloroglucinols hyperforin (36.5 ± 1.1 %) and adhyperforin (4.6 ± 0.1 %), the extracted phases contained mainly alkanes (predominately nonacosane – 39% of the GC-MS trace), fatty acids and wax esters. No components of higher polarity like flavonoids or anthraquinones were found and a broad variety of hyperforin oxidation products was detected. Most of the detected derivatives can be assigned to be epicuticular components. The GC-MS orientated analysis of isobaric wax ester isomers is described in detail and a rapid LC-DAD method for the quantification of hyperforin is presented.

P-64

K. Serdt, E. Lankmayr, J. Mocak, B. Balla, T. Wenzl, D. Bandoniene,

M. Gfrerer

UV-VIS, NIR, FTIR AND CHEMOMETRICAL CLASSIFICATION OF PUMPKIN SEED OILS

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The *goal of this work* is the *spectral* characterization of a variety of sorts of pumpkin seed oils, which together with the concomintant *sensory* characteristics and the *chemometrical* data processing facilitate to detect what physico-chemical properties are mostly informative with regard to the quality criteria of the oils.

Pumpkin seed oils enjoy special and increasing popularity mainly due to their characteristic taste especially in the Middle-European region. As they contain approximately 70 % of unsaturated fatty acids, a number of hydrocarbons, tri-terpenoides, carotenoides, tocopheroles and phytosteroles, they are important in global nutrition. The oil is not contained in the fruit, as e.g. in case of olive oils, but in the seeds, so that it cannot be obtained by mere pressing of the cold fruit but a denaturation of the proteins is required before pressing. Depending on the geographical conditions and due to a high content of very sensitive components as well as a rather complicated technology, various brands of pumpkin oil are produced in different quality.

The *sensory analysis* of the collected oil samples served for categorizing them into either 2 basic classes (fully satisfactory, not fully satisfactory) or 3 classes (excellent, satisfactory and bad quality samples). Further sub-classification of the bad quality samples was made according to their typical odour, taste and colour.

The *spectral characterization* of the oil samples was made by the UV-Vis, FTIR, NIR and partly also by the 3D-fluorescence spectra. The number of investigated samples was 70, 82, 80 for the mentioned three main kinds of the spectra, respectively, and their spectral signals were measured at 226 (UV-Vis) and 3464 (NIR) wavelengths, and 2592 wavenumbers (FTIR). In search for the *representative samples* of a good and a bad pumpkin oil, respectively, all good oil samples were sorted according to the decreasing absorbance value and their rank for all chosen wavelengths was found. Then the ranks were summed up for each sample and the representative sample was chosen according to the median rank sum value. The same was separately made for the bad oils. Surprisingly, the spectra of the representative good and bad oils were very similar in all examined spectral regions except the Vis part between 420–440 nm where the difference was more distinct.

The *chemometrical processing* of the spectral data was made by the *Principal Component Analysis* and mainly the *Linear Discriminant Analysis* (LDA) multivariate techniques, aiming to discover the relations between the spectral and sensory sample properties. In this study severe problems were caused by the collinearity of the variables (spectral wavelengths, wavenumbers). The key procedure for the exclusion of highly correlated variables from the original huge data matrices was based on the *Correlation Analysis* concerning all used variables for the given type of the spectrum. Only after this tedious procedure a further variable reduction step was possible by the stepwise backward selection method in the LDA.

The final six *chemometrical models*, calculated for each kind of the spectrum and two and three preselected pumpkin oil classes, enable a full discrimination of the pumpkin oils according to their sensory quality. In these models 52 (UV-Vis, 2 classes) to 62 (FTIR, 3 classes) optimally chosen variables (wavelengths/wavenumbers) were sufficient. The calculated models also enable a *prediction of the quality* of the yet unclassified pumpkin oil samples using only a small number of spectral variables.

P-65

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DSC OF EXPERIMENTAL HYLINE CARTILAGE DESTRUCTION CAUSED BY SEPTIC ARHRITIS IN RABBITS

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DSC of the hyaline cartilage is a new approach in investigating the degenerative processes of joints. With earlier experiments we could establish calorimetric standards of healthy hyaline cartilage both in human and experimental animal samples. Our studies in rabbits verified that experimentally induced degeneration of cartilage results in characteristic DSC scans, which differ from the healthy control ones.

In current experiment the New Zeeland rabbits (mean weight 3 kg) were kept under indifferent conditions for 2 weeks and were inoculated by Staphylococcus aureus OKI 112001 strain. This standard strain was isolated from wound [1] and cultivated on blood agar plate. Its identity was controlled by biochemical tests [2]. For the inoculation experiment the test strain was cultivated in Mueller Hinton broth (Oxoid Ltd. UK) [3] overnight at 37C and washed in physiological saline solution three times. At last the colony-forming unit (c.f.u.) of the test strain was checked by tube dilution method [4]: $8 \times 10^8 \pm 5\%$ c.f.u. 1.5 ml of this bacterial suspension was injected into the knee joint of back legs of rabbits. The injection was carried out day by day. We inoculated only the back legs of the animals. One leg was the untreated control. The duration of this experiment was 12 days from the first to the last injection. The first rabbit died on the 12^{th} day. At that time the rabbits involved into the experiment were sacrificed. The joints showed signs of inflammation macroscopically. We cultivated the fluid of joints on blood agar plates and could isolate the original inoculating *S. aureus* OKI 112001 strain from each knee joint.

The progress of sepsis was checked by SETARAM Micro DSC-II calorimeter. Uniform pieces of rabbit cartilages underwent a heating–cooling cycle in the 0-100 ºC temperature range with 0.3 K/min scanning rate. The DSC scans clearly demonstrated the development of sepsis from the first day of incubation compared to the healthy control.

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P-66

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QUANTITATIVE DETERMINATION OF α-LIPOIC ACID IN DIETARY SUPPLEMENT PREPARATIONS BY CAPILLARY ELECTROPHORESIS

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Dietary supplements attracted increasing interest in recent years. Since they are not regarded as drugs, they are not subject to the rules of drug control. Therefore there are not many determination methods existing for the quality control of such preparations.

α-Lipoic acid (6,8-thioctic acid) has been found as a gowth factor in diverse microrganisms and is present also on some plant and animal tissues. In earlier time it was regared as a vitamin, however, later it was found to be produced in the organism. It is known to act as a hydrogen-transferring cofactor and is involved in oxydative decarboxylation processes. The naturally occuring R(+) enantiomer is a prosthetic group for the pyruvate dehydrogenase complex. It pays an essential role in mitochondrial dehydrogenase reactions and has recently found considerable attention as antioxidant. It is used in therapy of diabetic polyneuropathy and in the treatment of heavy metal poisoning. Recently, it was found to show positive effects in age-associated neurodegenerative diseases and HIV infections.

This work deals with the development of a rapid method for the quantitative determination of lipoic acid in a dietary preparation by capillary electrophoresis (CE). Extraction from the tablets was carried out with methanol. After dilution with phosphate buffer pH 7, the samples were injected hydrodynamically for 6 sec. Background electrolyte was a phosphate buffer pH 7 containing 20% methanol. A SPECTROPHORESIS 1000 CE apparatus equipped with a UV-detector operated at 208 nm was used. The applied voltage was 15 kV. Analysis time was less than nine minutes. Quantitative evaluation was done by means of a calibration curve. Recovery was 98.5% with a precision of 2.8% RDS.

P-67

H.P. Strunk

CATHODOLUMINESCENCE SPECTROSCOPY IN AN ANALYTICAL TRANSMISSION ELECTRON MICROSCOPE: A POWERFUL TOOL IN SOLID STATE ANALYSIS

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Transmission electron microscopy is widely used in materials science, biology and medicine to analyze the structure of the crystalline and also of the amorphous states down to atomic dimensions. In the last two decades, analytical techniques have been added based on x-ray and on electron energy loss spectroscopy. These techniques permit the determination of the chemical composition within very small material volumes by the analysis of characteristic x-ray emission lines or energy loss shoulders, resp. Furthermore electron energy loss spectroscopy gives also access to the local (joint) density of states by evaluation of the very low energy loss spectral properties and, by using the Kramers-Kronig relation, of the dielectric function. What such an equipment is missing is the direct and very informative access by optical spectroscopy. We have in recent years therefore attached to our analytical transmission electron microscope a cathodoluminescence spectrometer that, in addition to all other signals mentioned before, also delivers the optical response of the electron-irradiated site (exception are metallic materials) with dimensions down to nanoscopic dimensions. This spectrometer can analyze spectra from infrared to ultraviolet (1800 to 180 nm) with around 1 nm wavelength resolution, the specimen temperature can be varied between 10 and 300 K (and even higher), without sacrificing the double tilt goniometer function of the specimen holder necessary for crystallographic and defect analysis.

This contribution presents selected examples taken from our institute's work on optoelectronic and photovoltaic materials where cathodoluminescence considerably contributed to the understanding of electronic properties. We are now able to display nanometer thick quantum well layers by their light emission and can analyze composition and thickness variations on a nanometer scale. Thin Copper Indium-Diselenide photovoltaic films are micro-crystalline and exhibit considerable differences from grain to grain as is concerned the recombination of minority carriers. These ca now be investigated by means of cathodoluminescence. In all cases imaging of the samples using light of accordingly selected wavelength ('mapping') helps in visualizing the local materials properties. This broad microanalytical approach can also be of value for the investigations of other luminescent systems such as fluorochromes.

P-68

Z. Szabó, E. Ősz, T. Lóránd

MS AND NMR INVESTIGATION OF BIOACTIVE QUINAZOLONES

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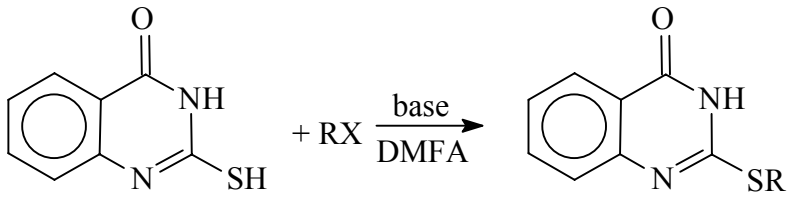
In order to prepare effective PARP [poly(ADP)-ribose)polymerase-1] inhibitors, starting from 2-mercapto-4(3H)-quinazolone (**1**) several derivatives - 2-alkylsulphanyl-3H-quinazolin-4-ones (**2-12**) - have been synthesized using a simple alkylation method. Some of them are known compounds. Their structure was studied by MS and NMR methods.



1 **2-12**

R = ethyl, n-butyl, allyl, propargyl, etc.

The structure elucidation of compounds **2-12** was performed by NMR measurements. NMR spectra were recorded with Varian ^{UNITY}*INOVA* 400 WB (400/100 MHz for $^{1}H/^{13}C$) spectrometer. The ^{1}H and ^{13}C assignments were based on ^{1}H-^{1}H COSY, gradient enhanced ^{13}C-^{1}H HSQC and ^{13}C-^{1}H HMBC experiments executed using standard Varian software. Structural evaluation of quinazolones by mass spectrometry has been carried out by atmospheric pressure chemical ionisation interface (APCI). This way of ionisation seems to be quite powerful providing us with enough structural information to be capable of identifying our compounds. All samples were dissolved in methanol and injected hydrodinamically. The data acquisition occurred simultaneously by changing the fragmentation voltage therefore we could get molecular peak, molecular radical peak and their fragments. Obviously, other important parameters, as well as probe temperature, discharge voltage were also optimised.

P-69

K. Szabó, N. Marek

EFFECT OF PERCHLORATE ION ON THE FLUORESCENCE PROPERTIES OF THE PROTONATED 2,2'-BIPYRIDINE

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Interaction between the monocation of an aromatic amin, the 2,2'-bipyridine and perchlorate anion was studied. The protonation process by perchloric acid has been followed by UV absorption spectra. The relative fluorescence intensity decreased parallel with the formation of protoncomplex.

In order to study the effect of perchlorate anion, lithium perchlorate of increasing concentrations was added to the aqueous solution of monocation. The $BpH^{+}\cdot ClO_4^{-}$ ionpair has been formed as a result of the interaction between monocation and lithium perchlorate. The latter is frequently used as a background electrolyte when studying at the equilibria in solutions. Rotation of the protoncomplex is inhibited by the ionpair formation and the rigid molecule structure results in a significant increase of the fluorescence intensity.

P-70

S. Szakály, G. Óbert, B. Schäffer, D. Lőrinczy

ISOTHERM CALORIMETRIC EXAMINATION OF PROBIOTIC BACTERIA OF MILK DRINKS PRODUCED BY VARIOUS (CO-FERMENTED AND POST-INOCULATED) METHODS

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In the last decade probiotic milk drinks (such as the Japanese YACULT) have enjoyed steadily increasing sales worldwide, the singular function of these products being that consumption of a daily amount (75 ml) will maintain a balance in intestinal flora, in the sense that primarily probiotic bacteria become preponderant. Hungarian Dairy Research Institute (HDRI) has developed probiotic milk drinks by two different technologies based on cultures of Prebiolact-2 (which it isolated in the course of a joint international project), Lactobacillus casei and Bifidobacterium bifidum. When fermented together a greater live bacteria titer could be obtained ($1x10^9$/g), while in production by the two-stage technology a relatively lower live bacteria titer ($2x10^7$/g) was accompanied by a somewhat higher total titer of probiotic (live+dead) cells ($1.2x10^9$). As the human clinical examinations show, the milk drinks produced by the two-stage (post-inoculated) technology have a more favorable influence on intestinal flora than the one-stage (co-fermented), the proliferation capacity of microbes in milk drinks made by two different technologies was examined.

For the examinations 5% probiotic milk drinks produced by the one-stage and two-stage technologies were suspended in non-fat sterilized milk, then fermented at 37°C until pH 4.7 was reached: in other words, a milk drink ferment was made. For the isotherm assays approx. 450 mg sterile non-fat milk and approx. 50 mg milk drink ferment were separately put into a mixing batch vessel and left at 37°C until thermal equilibrium was reached. Next the milk drink ferment was injected into the milk, and microbe proliferation was recorded for 12 hours by a SETARAM Micro DSC-II calorimeter at 37°C under isothermic conditions.

From the deconvolution analysis of the heat flow-time curves it was established that the microbes of products produced by both technology proliferate well, but the proliferation of Bifidobacteria in probiotic milk drinks produced by the two-stage technology was more pronounced than that of produced by the one-stage method.

P-71

S. Szakály, B. Schäffer, D. Lőrinczy

IMPACT OF THE RATIO OF PROBIOTIC MICROBES IDENTIFIABLE BY ISOTHERM CALORIMETRY ON THE SENSORY AND HUMAN PHYSIOLOGICAL CHARACTERISTICS OF PROBIOTIC KEFIR (BIOFIR®)

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Kefir, though known to be a healthy food, shows no probiotic characteristics, thus in order to make it probiotic it is necessary to use a probiotic culture in addition to the kefir fungi during fermentation. The question, however, is whether the two ones could proliferate in a mixed environment, as the optimal temperature for the kefir fungi is a relatively low (18-20°C), and is 36-45°C for the thermophilic probiotic culture. Sensory and microbiological examinations show that a shared fermentation temperature cannot be established. Therefore, Hungarian Dairy Research Institute (HDRI) has elaborated a production procedure in which the basic fermentations are conducted separately and are not united until a specific point in the technology. In the final product (Biofir®) the characteristics of the kefir were determined organoleptically, while the presence of probiotic bacteria by isothermic calorimetry and its probiotic effect by human clinical examination.

In order to indicate the probiotic microbes 2% Biofir was suspended in non-fat sterile milk and the suspension was fermented at 37°C (the fermentation temperature of the probiotic culture) until pH 4.7 was reached: in other words, a Biofir ferment was produced. For the isotherm assays approx. 450 mg sterile non-fat milk and separately approx. 50 mg Biofir ferment were put into a mixing batch vessel and left at 37°C until thermal equilibrium was reached. Later the Biofir ferment was injected into the milk, and microbe proliferation was recorded for 12 hours by a SETARAM Micro DSC-II calorimeter at 37°C under isotherm conditions. For the human clinical examination a total of 75 hyperlipidemic adults were choosen, of whom 15 consumed traditional kefir and 60 Biofir for 4 weeks. At weeks of 0^{th}, 2^{nd} and 4^{th} 22 parameters of the participants' blood were measured as well as the microbiological condition of their stool.

By analysing the heat flow-time curves it was determined that the probiotic bacteria proliferates well in Biofir. The human clinical examination shows that when traditional kefir was consumed there were no significant changes in the parameters measured from the blood and stool. In contrast, consumption of Biofir:

- reduced the level of total cholesterol in the blood,
- increased the ratio of beneficial HDL cholesterol and reduced that of harmful LDL,
- substantially reduced the blood triglyceride level,
- increased the probiotic cell titer in the stool by several orders of magnitude, and
- to an extremely high extent (by a factor of nearly 60) increased the Bifidobacteria titer in the stool.

P-72

Z. Szántó, L. Benkő, B. Gasz, G. Jancsó, E. Rőth, D. Lőrinczy

DIFFERENTIAL SCANNING CALORIMETRIC EXAMINATION OF THE TRACHEAL CARTILAGE AFTER PRIMARY RECONSTRUCTION USING DIFFERENT SUTURE TECHNIQUES

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Introduction: Resection and subsequent end-to-end anastomosis of the windpipe is a tried-and-tested acceptable method for the surgical treatment of segmental defects. There are a variety of different techniques for tracheal end-to-end anastomosis, but controversial reports highlight the fact that the suturing technique in the anastomosis is still subject to debate. We aimed to show the intra-operative effect of the continuous and simple interrupted suturing technique on the microcirculation as well as the postoperative changes of the tracheal cartilage using differential scanning calorimetry.

Materials and methods: Resection and subsequent reanastomosis of the cervical trachea was performed in 14 adult beagle dogs (average 17 kg). The trachea was anastomized with continuous (group I, n=7) and simple interrupted (groupII, n=7) sutures (PDS 6/0). Laser Doppler measurements were performed to detect possible alteration of the local microcirculation. The animals were sedated and painlessly euthanized after a 20 days of follow up. Rings of the anastomotic area were subjected to calorimetric measurement by a SETARAM Micro DSC-II calorimeter.

Results and discussion: Results depict no change after the resection of the trachea, but significant decrease following the completion the anastomosis with continuous sutures. According to the present study the thermograms may prove the presence of structural changes of the cartilage in the short-term follow up (smaller melting temperature and calorimetric enthalpy in the operated dog). The differences were clearly demonstrated between the intact cartilages and the ones involved in the anastomosis.

P-73

K. Szarka, E. Bódis, M. Nyitrai and B. Somogyi

THE EFFECT OF TROPOMYOSIN AND MYOSIN S1 ON INTERMONOMER FLEXIBILITY OF ACTIN FILAMENT

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Flexibility measurements were carried out on actin filament in the presence and in the absence of tropomyosin and myosin S1. Actin filaments were polymerized from IAEDANS (donor) or IAF (acceptor) labelled monomers in a given concentration. Both dyes bind to Cys374 amino acid on actin selectively. FRET efficiency was determined between donor and acceptor pairs and intermonomer flexibility was measured according to Somogyi et al (1984). It was shown that the flexibility of actin filaments became more rigid in the presence of tropomyosin than in the absence of the protein while in the presence of myosin S1 the filament became more flexible than in the absence of S1.

P-74

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HPLC-RIA ANALYSIS OF STEROID HORMONE PROFILE IN A VIRILIZING OVARIAN TUMOR

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Ovary is the major source of sexual steroid hormones in the female organism. Ovarian tumors, in most cases, feature exaggerated steroidogenic activity, associated with elevated steroid hormone levels in blood and severe endocrine manifestations. Steroid secretion depends on type and progression of the tumor: most often estrogens, but occasionally androgens and exceptionally progestagens or corticosteroids are overproduced. Blood serum steroids or urinary metabolites do not reflect to the ovarian steroidogenesis. Primary steroid biosynthesis in the ovary can be examined via determination of the steroid concentrations in the tissue. Tissue sample, however, is a complex matrix containing large amount of lipid substances and cross-reacting steroid compounds. Therefore, quantitation of intratissular steroids requires highly specific and meticulous analytical methods.

For measurement of ten key steroid hormones in an ovarian tumor tissue we developed an analytical method based on liquid and solid phase extraction, high-performance liquid chromatography (HPLC) separation and radioimmunoassay (RIA) quantification. Tissue specimen was minced, and then extracted with ethyl acetate using ultrasonic homogenization. Solvent was evaporated under nitrogen and the extract was purified on a 500 mg C_{18} mini-column with methanol-water eluents. Steroids were isolated by reversed phase HPLC, on a 250 x 4.0 mm C_{18} column with 55 v/v% methanol-water eluent. Fractions were collected and the eluted steroids were detected by radioactivity of tritiated internal standard steroids added to the tissue sample before the extraction procedures. Fractions of isolated steroids were dried and dissolved in RIA buffer. RIA samples were incubated with specific antisera and tritiated tracers for 18 hours at 4 °C. Free fraction of steroids was eliminated by adsorption on dextran coated charcoal and radioactivity of bound fraction was measured. Steroid content of the tissue sample was calculated with consideration of recovery of labeled internal standards and methodological background.

Stromal tumor of ovary was obtained surgically from an 18 years old female patient with extremely high androst-4-ene-3,17-dione (10.3 ng/ml) and testosterone (3.8 ng/ml) blood serum levels. In the tumor specimen very high 17alpha-hydroxyprogesterone (2090 ng/g), dehydroepiandrosterone (830 ng/g), androst-4-ene-3,17-dione (860 ng/g), testosterone (1650 ng/g) concentrations, and less progesterone (102 ng/g), and androst-5-ene-3beta,17beta-diol (92 ng/g) were determined by the HPLC-RIA method. Tissue levels of 5alpha-dihydrotestosterone, 5alpha-androstane-3alpha,17beta-diol, 5alpha-androstane-3beta,17beta-diol, and 17beta-estradiol were found to be 19.0, 5.8, 8.1, and 3.3 ng/g, respectively.

Steroid profile analysis verifies a pathological ovarian steroid biosynthesis and suggests that activity of delta5-3beta-hydroxysteroid dehydrogenase, converting 3beta-hydroxy-5-ene-steroids to 3-oxo-4-ene-steroids, is particularly elevated in this tumorous tissue. Different proportions of steroids in tissue vs. serum are consequence of peripheral conversion and highlight the importance of the measurement of steroid hormone concentrations in the tissue itself. Present data demonstrate that analysis of intratissular steroid profile by an HPLC-RIA method offers an adequate tool for studying steroidal pathophysiology of endocrine organs and tumors with presumed steroidogenetic activities.

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LUMINESCENCE BASED DETERMINATION OF URANYL ION IN ENVIRONMENTAL SAMPLES

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The analytical research in uranyl ion determination is focused on the development of new preconcentration materials and on-field measurement techniques. Electrochemical techniques and a wide variety of optical methods, such as absorption, different kind of luminescence measurements, optical chemical sensors are usually used for uranyl determination in aqueous and environmental samples. Inorganic and organic materials present in an environmental sample could affect the luminescence properties of uranyl. The fluorescence emission of humic acids and other organic substances overlaps the emission of uranyl ions. Dissolved inorganic ions generally quench the emission of uranyl. Different kind of extraction methods, selective complexing and masking agents are usually used to separate the uranyl ions.

In this work the use of sodium pyrophosphate as a luminescence enhancer is discussed. A continuous wave Jobin-Yvon τ-3 fluorometer and a Perkin-Elmer 50 fluorometer equipped with phosphorescence unit were used for the experiments. Steady-state luminescence intensity as well as life-time measurements were carried out to find the optimal chemical conditions - such as pH and ionic strength – for the analysis. Since the excited-state lifetime of uranyl is sensitive to changes in pH and ionic strength the optimal experimental setup (gate- and delay times, etc.) with respect to future laser induced measurements are also reported. The dynamic range and sensitivity of the method are also shown.

A procedure for uranyl determination in real environmental samples was developed. The results obtained by our method as well as a comparison with standard luminescence based techniques are also given.

P-76

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INDICATION OF PROBIOTIC MICROBES IN PROBIOTIC CHEESE SPREADS BY ISOPERIBOLIC BATCH CALORIMETRY

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The probiotic cheese spreads developed by the Hungarian Dairy Research Institute (HDRI) have a better Ca:P ratio than traditional brands and spread easily and without sticking even cold; further, their live lactic acid bacteria content gives them additional advantages as well. These advantages derive from the fact that a probiotic culture may also be employed during fermentation, through which the cheese spread may become probiotic if the culture proliferates. The international specification is a probiotic cell titer of at least 10^6/g. However, during fermentation the probiotic cheese spread must also use other non-probiotic cultures to produce the right taste. Given that the microorganisms in the cultures are all cocci, their indication in a mixed environment is difficult and time-consuming. It appears possible, however, to apply the isoperibolic batch calorimetry method due to their differences in heat production.

Prior to the examinations probiotic microbes were grown in probiotic cheese spreads by mixing 5% from them into non-fat, sterile milk, then fermenting them at 30°C (the fermentation temperature for probiotic cheese spread) until pH 4.7 was reached: in other words, cheese spread ferment was produced. For the heat flow measurement approx. 450 mg sterile non-fat milk and approx. 50 mg cheese spread ferment were separately put into a mixing batch vessel and left at 30°C until thermal equilibrium was reached. Later the cheese spread ferment was injected into the milk, and microbe proliferation was recorded for 16 hours by a SETARAM Micro DSC-II calorimeter at 30°C under isotherm conditions. Total plate titer was determined as described above, but using a thousand times greater quantities. By the method described the isothermic DSC curves of the probiotic culture used in the production of probiotic cheese spreads and of butter culture samples were recorded, and total plate count was also determined.

In order to analyze the heat flow-time curves a deconvolution was performed using Gaussian curves. It was confirmed that probiotic bacteria proliferated in the probiotic cheese spreads, and their ratio was greater than 50%, with a total plate titer of $3x10^7$/g. Accordingly, the probiotic cheese spread developed by HDRI contains an order of magnitude more probiotic bacteria than the internationally accepted cell titer of 10^6/g.

P-77

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IMPACT OF THE RATIO OF EXOPOLYSACCHARIDE- (EPS)-PRODUCING MICROBES IDENTIFIABLE BY ISOPERIBOLIC BATCH CALORIMETRY ON THE FUNCTIONAL PROPERTIES OF HEAT-RESISTANT SOUR CREAM

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The spread of sour cream production from homogenized cream in the 1970's dramatically improved the cost-effectiveness of production and the quality of the product. The improvement in quality was signified by the firm texture of the sour cream, its resistance to syneresis, its whitening ability and the increase of shelf-life. The only product characteristic which was diminished was its heat resistance; that is, it precipitated in hot food. The Hungarian Dairy Research Institute (HDRI) has elaborated a technology which eliminates this one disadvantageous characteristics. One element of the technology is the use of exopolysaccharide- (EPS)-producing lactic acid bacteria. However, as this lactic acid bacteria produces no aroma, aroma-producing lactic acid bacteria must also be used during production in order to ensure the characteristic flavor of sour cream. Since the proliferation optima of EPS-producing and aroma-producing lactic acid bacteria cultures do not coincide, there is a question of the ratio at which the microbes of the individual cultures are able to proliferate at a shared fermentation temperature. The answer to this question was sought by isothermic calorimetry.

For the experiments cream with 20% fat treated by the method described in the HDRI technology was sterilized and approx. 450 mg was poured into a mixing batch vessel. Approx. 50 mg culture was poured into the injection cylinder, which was either an EPS- or aroma-producing lactic acid bacteria culture, or both in a 50-50% mixture. This was left at 30°C until thermal equilibrium was reached, then the solvent was injected into the cream and the heat flow time curve of microbe proliferation was recorded for 12 hours by a SETARAM Micro DSC-II calorimeter under isothermic conditions.

In an analysis of the heat flow curves by a deconvolution it was determined that

- both lactic acid bacteria cultures proliferate well at the non-optimal temperature of 30°C,
- proliferation of the thermophilic EPS-producing culture was faster than that of the mesophilic aroma-producer,
- the two cultures do not inhibit each other in mixed culture, and
- as determined by the deconvolution the ratio in mixed culture was 75% EPS-producing and 25% aroma-producing.

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A NEW OPTION OF EXAMINING THE HUMAN HYALINE CARTILAGE: DIFFERENTIAL SCANNING CALORIMETRY

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Objective: Arthritis of major joints, especially osteoarthritis of the knee is a very frequent disease of human beings mainly in the developed countries, major pathological changes occuring in the hyaline cartilage. The pathology of osteoarthritis has been subject of many publications before, using a wide spectrum of examining methods. The aim of our investigation was to introduce a new method in cartilage research and to prove its capacity to study the human hyaline cartilage.

Design: In a cooperation of three institutes, the authors examined knee joint hyaline cartilage with a method never applied before for such targets: differential scanning calorimetry is a thermodynamic method, which has been established for decades in the research of biological systems.

Results: Examination of samples of cadaver origin, judged to be intact cartilage, proved that calorimetry can be applied in cartilage research. The research group set the calorimetric standards for healthy human knee joint cartilage, demonstrated thermal differences between intact and arthritic samples as well as differences between various stages of osteoarthritis.

Conclusion: Besides explaining possible causes for experienced thermodynamic effects, the authors reflect upon future research ways and the possibilities of applying the method in practice.

P-79

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DETERMINATION OF HAIR DYE METABOLITES IN ANIMAL LIVER CELLS AND HUMAN KERATINOCYTES BY HPLC/MS

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Prediction of metabolic pathways of hair dyes after their application is an important aspect to meet international safety requirements of cosmetic products. Because of ethical reasons and general prohibition of animal tests for cosmetic products, two alternative testing systems e.g. S9 rat liver homogenisate induced by 3-methylcholanthrene and human keratinocytes were introduced to imitate metabolism in human body. The substances analyzed were the two oxidative hair dyes 4-amino-m-cresol and 5-amino-o-cresol.

With respect to MS investigations to be performed various isocratic mobile phase compositions and gradient systems were tested by HPLC/DAD. As the application of solvent mixtures is somewhat limited for MS, mobile phases containing acetate buffer and organic solvents were used for optimization. Comparing different stationary phases a polymeric RP8 Waters XTerra® column allowing the use of pH values up to 12 proved to be most suitable. For the separation of metabolites in S9 an isocratic mobile phase consisting of acetate buffer pH 8.7 and methanol, 80:20 for 4-amino-m-cresol and 78:22 for 5-amino-o-cresol, was applied in combination with MS analysis using APCI. A number of oxidation and conjugation products could be detected and identified by their characteristic m/z ratio.

For the metabolism study with HaCaT a multi-step gradient system containing acetate buffer pH 8.7 and methanol as well provided satisfying resolution of the peaks obtained. Due to a different enzyme pattern and a minor metabolic enzyme content of HaCaT compared to S9 only the N-acetate derivative could be identified as conjugation metabolite.

P-80

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EXTRACTION OF ANTIOXIDANTS FROM PP AND PE

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In order to improve the physical properties and to extend the lifetime of synthetic polymers, most polymeric materials contain additives, such as antioxidants, UV stabilizers, metal deactivators as well as slip-, antiblock- and antistatic agents. As the purity and amount of additives incorporated into a polymer product affect the properties of the polymer, there is a need for reliable and rapid analytical methods to determine the amount of additive present. Quantitative polymer/additive analysis is costly and needs to be considered carefully both in terms of time efficiency and reliability of the results, in particular in industry for quality control purposes. Beside the other disadvantages (long extraction times, large extraction solvent demand) Soxhlet extraction, still the standard extraction method in many industrial norms, applies temperatures below the boiling point of the chosen extraction solvent. It is well known, that diffusion rates an increase in temperature and swelling the polymer by using stong solvents Microwave-assisted extraction using pressurized closed vessels enables to extract up to 8 samples simultaneously at high temperatures, in rather short extraction times and fully automated. In the present study microwave-assisted extraction was investigated for the extraction of the antioxidants Irganox1010, Irgafos168 and butylhydroxytoluene (BHT) from polyethylene and polypropylene samples. The analysis of the antioxidants was performed by reversed phase HPLC-UV as well as HPLC-DAD.

P-81

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DETERMINATION OF CLONIDINE IN HUMAN PLASMA SAMPLES BY MEANS OF GC-MS

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Clonidine, an α-sympathomimetic agent, stimulates α-adrenergic receptors, whereas its effect on α_2-receptors is stronger than on α_1-receptors. Mainly clonidine was used in the treatment of hypertension, but recent studies show that clonidine also possesses analgetic effects. In this context plasma samples of patients medicated with clonidine against the pains after arthroscopical knee joint surgery had to be analysed. In order to be able to determine the expected low concentrations of clonidine a sensitive GC-MS method had to be developed.
Quantitative analysis was carried out by GC-MS after appropriate sample preparation using clonidine-d_4 as internal standard. The preparation included protein precipitation with tetrahydrofuran and sodium chloride, solid-phase-extraction with cartridges containing divinylbenzene and N-vinylpyrrolidone and derivatization of clonidine and clonidine-d_4 with 2,3,4,5,6-pentafluorobenzylbromide. After liquid-liquid-extraction with toluene, diisopropylether, dichloromethane and water the samples were concentrated in toluene and dichloromethane and analyzed in the GC-MS System using electron impact mass spectrometry (70eV) and Single Ion Monitoring (SIM). Using this method clonidine could be determined in human plasma samples with good reproducibility and a detection limit of 60 pg/ml.

P-82

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PRE-CLINICAL METHODS FOR THE DETERMINATION OF INSULIN SENSITIVITY

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We compared the hyperinsulinaemic euglycaemic glucose clamping (HEGC) procedure and the rapid insulin sensitivity test (RIST) to characterize insulin sensitivity in anaesthetized rats. The changes in insulin sensitivity were then validated by means of direct measurement of insulin-stimulated glucose uptake using tissue accumulation of radioactive 2-deoxy glucose in skeletal muscle samples obtained from the animals undergone either procedure. The two methods were compared in terms of comparing the maximum increase in insulin sensitivity achieved by activation of the most potent endogenous insulin sensitizing mechanism ie. the hepatic insulin sensitizing substance (HISS) mechanism. The HISS mechanism is known to be sensitive to nitric oxide (NO) synthase inhibitors and to be activated by insulin. The tissue insulin sensitivity is increased through the release of a currently undefined substance to the circulation from the liver termed HISS that increases the sensitivity of peripheral tissues to the hypoglycaemic effect of insulin. In case of the HEGC method, insulin was infused to attain a stable high plasma insulin immunoreactivity of 100 μU/ml determined by radioimmunoassay, as for the RIST method, the HISS mechanism was activated by a 50 mg/kg i.v. insulin bolus. Euglycaemia was kept constant by means of glucose infusion. With the HEGC and the RIST methods, insulin sensitivity was defined as the average rate of glucose infusion and the amount of glucose/kg body weight/40 min (RIST index) infused, respectively, to maintain an euglycaemic state. During HEGC 16±4.2 mg/kg/min glucose was able to maintain euglcaemia, which decreased to 8±2.9 ($p<0.05$) after administration of 10 mg/kg N^G-nitro-L-arginine methyl ester (i.p.), a NO synthase inhibitor. The RIST index decreased by 55±6.9 % ($p<0.05$) after L-NAME. Similarly, the 2-deoxyglucose uptake by the gastrocnemius muscle was decreased by 49.9±5.8 ($p<0.05$) and 52.3±7.4% ($p<0.05$) with the HEGC and the RIST methods, respectively. The results show that both the HEGC and the RIST methods supplemented with tissue radioactive 2-deoxy glucose uptake determinations are appropriate methods to determine tissue insulin sensitivity in whole animals.