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Werner Prize **1**

From Simplicity to Complexity *via* Subcomponent Self-Assembly

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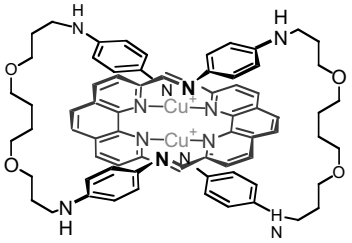
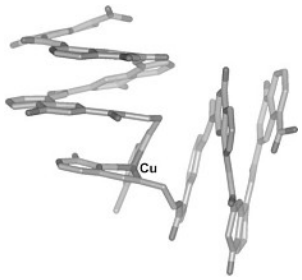
The creation of complex structures from simple building blocks *via* thermodynamic self-assembly requires an understanding of the rules followed during the self-organization process. Since the inception of this research program in August 2003, we have sought to identify these rules and to apply them to synthetic problems. "Subcomponent self-assembly"[1] describes systems in which simple amines and aldehydes may be induced to come together around metal-ion templates *via* the formation of imine (C=N) bonds.[2,3] We have thus been able to create complex structures such as a catenane (below, right)[4] or a pair of helical molecules linked at a right angle through metal coordination (below, left), having a tertiary structure similar to that of a protein. These structures are capable of dynamic reassembly at both N→Metal and C=N linkages.[5]





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Gramaticakis – Neumann Prize 2007 **2**

Photoinduced functions in multicomponent molecular systems

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Light-induced processes are at the basis of fundamental natural phenomena as well as of a variety of applications. Since the functions that can arise from the interaction between light and matter depend on the degree of complexity and organization of the receiving 'matter', the research on these processes has progressively moved from molecular to supramolecular (multicomponent) systems. Examples of multicomponent systems capable to perform specific functions under light stimulation (photochemical molecular devices, PMDs) have been developed [1], relying on processes such as photoisomerization and photoinduced electron or proton transfer.

Here we will describe a few recent examples of PMDs studied in our laboratories [2], designed to (i) process binary information (molecular logic gates and circuits) or (ii) undergo controllable motions of some molecular components with respect to the others (molecular machines).

Apart from futuristic applications, investigations on PMDs can increase the basic understanding of a variety of processes, as well as develop reliable theoretical models. This research has also the important merit of stimulating the ingenuity of chemists, thereby instilling new life into chemical sciences.

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Analytical Chemistry **3**

"Snapshots" of a heterogeneous catalyst at work: From integral towards spatially resolved spectroscopic monitoring of solid materials

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To gain information on the structure of solid materials such as heterogeneous catalysts, *in situ* X-ray absorption spectroscopy (XAS) is a well-suited technique. In certain processes the structure of a catalyst may vary along a catalytic reactor particularly if prominent gradients in temperature or concentration occur. The investigation of these phenomena requires spatially resolved techniques on a microscale.

Here we present structural data of noble metal catalysts during partial oxidation of methane (CPO). To efficiently record XAS spectra in a locally resolved way, a new approach is suggested using a CCD-camera [1]. The results are compared to those from μXAS-measurements. In addition, the temperature gradient was determined using an infrared camera [2]. A correlation between the structure of the catalyst, the catalytic data and the axial temperature profile in the catalytic reactor was established. Tremendous structural changes of the noble metal particles within a gradient of less than 100 mm thickness and a strong dependence of the gradient on the reaction conditions (temperature, space velocity) were observed. Very recently, "snapshots" were not only taken of the variation in catalyst structure and temperature, but we even succeeded in following these changes in a time-resolved manner using a CCD- and an IR-camera.

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Analytical Chemistry **4**

Chemometric tools to simplify method development: screening of doping agents in urinary samples.

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Analyses in anti-doping control occur in two steps. First, a generic fast screening analysis is used to determine the presence of a large number of forbidden compounds in urine. Second, in the case of positive result, a specific procedure (confirmatory analysis) has to be performed to quantify the substance(s).

Screening method development is tedious and time consuming due to the necessity to optimize the sample preparation of a large quantity of compounds. The use of chemometric tools is therefore proposed to reduce the number of tested analytes in method development by choosing representative compounds of the whole set.

In this study, a group of thirty-six doping agents consisting of diuretics and beta-blockers was used. A standard solution containing all analytes was loaded, washed and eluted with four different SPE sorbents. All fractions were collected and each compound was retrieved by LC-ESI-MS. In order to bring out differences among compounds, a multivariate analysis approach was used. A small number of groups emerged and subsequent method development was performed by selecting representative compounds in the obtained clusterisation.

Analytical Chemistry **5**

Microchip electrophoresis bioanalytical applications

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The implementation of microfabricated separation devices (so called microchips) in electrophoresis has become a trend in modern separation science. However, the miniaturization of the separation to microchip brings along some benefits (speed, negligible consumption of substances or high throughput analysis) also some drawbacks (limited separation efficiency and selectivity, higher detection limits or the absence of automation).
Nevertheless, in some application areas the advantages of the microchip separations overcome the limitations. A development of a selective and sensitive detection, often involving expensive reagents, or a fast development of a separation method could be listed as the examples of such applications. Specific measurements for particular purposes (affinity measurements and other) represent then further useful applications of microchips
The listed applications areas of microchips will be demonstrated by presenting the results of our research. The sensitivity of amperometric detection of catecholamines has been specifically enhanced with the help of enzymes [1] and also carbon nanotubes [2]. A complex separation method has been developed for simultaneous separation of all three catecholamines and their cationic metabolites [2]. The separation method together with the developed sensitive detection was then successfully applied for the measurement of the level of these compounds in the mouse brain sample [2]. A specific application of the microchips will be represented by isoelectric focusing measurements on a microchip, which are a subject of our ongoing research. Using a whole-column imaged optical detection with the microchip, a credible determination of the isoelectric points of selected proteins by isoelectric focusing was feasible.

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Analytical Chemistry **6**

Development of a method to characterize nanoparticle uptake into Corn plants

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Nanoparticles (NP) are increasingly used in various applications e.g. as catalysts or milling agents. Therefore risks of exposure during production processes and distribution in the environment require clarification about the potential risks. Studies using human lung cells have shown that agglomeration, sedimentation and diffusion of NP affect uptake by cells [1]. This study has investigated the uptake of CeO_2 NP during exposure to airborne NP into Corn plants.
For the experiments, the plants were placed in a glove box that allowed the production of NP flame spray synthesis in situ for a short duration (~1 min). The NP are distributed within the glove box by a fan. The plants remain inside the glove box for a certain time before determining the amount of CeO_2 that was taken up by the plant cells. Due to the low amounts that are taken up by the plants, inductively coupled plasma mass spectrometry (ICP-MS) is used to determine Ce-concentrations in the plant material. Sample preparation includes a washing step in order to discriminate between NP that were enter the cells form surface contaminants. Details of the washing procedure and results optained will be discussed in this presentation.

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Analytical Chemistry **7**

Tandem Mass Spectrometry Data Mining: Product Ion Abundances Provide Complementary Peptide and Protein Structural Information

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Revealing the information hidden in the product ion abundance distribution in tandem mass spectrometry in general and in electron capture/transfer dissociation (ECD/ETD) in particular could provide substantial benefits for peptide and protein structural analysis and improve fundamental understanding of gas-phase radical ion chemistry during ECD/ETD. Here we report on a recent progress in this direction based on a novel insight into correlation of ECD/ETD radical and prime product ion yield with solution-phase peptide and protein secondary structure.

First, comparison of radical/prime product ion abundance ratio in ECD versus activated ion (AI)-ECD demonstrates a strong and reproducible dependence on ion internal energy. This dependence allows for product ion type (N-terminal or C-terminal) determination in many cases greatly facilitating *de novo* peptide sequencing and protein identification.

Second, targeted analysis of ECD/ETD product ion abundances of several peptides reveals a qualitative correlation between product ion yield and amino acid hydrophobicity. For linear peptides it opens the way toward sequence-based prediction of ECD fragmentation patterns. Current status of method validation on selected peptide libraries will be presented.

Furthermore, ECD product ion distribution correlates with suggested solution-phase secondary structure of several peptides and protein enzymatic fragments, particularly for alpha-helical structures. Mechanistic studies in this area are directed toward improving our understanding of the ECD process and progressing toward possible site-specific secondary structure inferred from ECD data.

Analytical Chemistry **8**

Imaging and chemical analysis of biological nanostructures using a setup for tip-enhanced Raman spectroscopy (TERS)

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Biological samples can be highly heterogeneous on the nanometer scale. Bacterial adhesion and biofilm formation, for example, are controlled by nanometer-sized structures, such as pili, flagella, and extracellular polymeric substances (EPS). The arrangement of the different EPS (e.g. polysaccharides, proteins, humic substances) in the biofilm matrix is largely unknown, because microscopy techniques used so far were restricted in spatial resolution to the micrometer and higher nanometer range (e.g. confocal laser-scanning microscopy, CLSM) or did not provide any chemical information (e.g. atomic force microscopy, AFM). A better insight into the distribution of the different extracellular nanostructures is important for the improvement of biocides as well as for process optimization in wastewater treatment.
A combined CLSM-AFM setup allows AFM imaging of selected areas of a CLS micrograph with nanometer-scale resolution revealing bacteria, pili, flagella, and EPS. For nanometer-scale chemical analysis, the feasibility of tip-enhanced Raman spectroscopy (TERS) was demonstrated, where Ag-coated AFM tips in the laser focus enhance the Raman signal, yielding chemical information with a lateral resolution of down to 20–50 nm. Alginates in the form of nanometer-sized fibers and hydrogels were used as model samples for biofilms and investigated successfully by AFM and TERS. Other biochemical compounds under study are proteins and lipids.
The goal of further studies is to improve TERS to become a robust tool for the analysis of biological samples, which allows, for example, the elucidation of the distribution of different biopolymers (e.g. polysaccharides and proteins) inside the biofilm matrix and can be applied in many other fields of biology and medicine.

Analytical Chemistry **9**

Lipophilicity index measurement of polar compounds using hydrophilic interaction liquid chromatography

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Lipophilicity is one of the most used physico-chemical parameters to build structure activity relationships (SAR). Methods to measure, with a good confidence level, the lipophilicity of very polar compounds are still missing except the obsolete thin layer chromatography (TLC) [1].
In this study, the retention factors (log k) of 26 neutral polar compounds were measured using hydrophilic interaction liquid chromatography (Hilic), and were evaluated as lipophilicity indices to measure log P_{oct}. A linear solvation energy relationships (LSERs) analysis was performed to elucidate the intermolecular forces controlling the retention in Hilic.

The relative contribution of each parameter, obtained by standardization, shows that significant differences lies between partitioning in n-octanol/water system [2] and Hilic interactions. The H-bond donor acidity parameter (α), is particularly more important in Hilic.
The results show that log k in Hilic is not a parameter of choice to measure the log P_{oct} of polar compounds. However, these retention factors provide interesting information which could be used as a lipophilicity/polarity parameter in SAR.

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Analytical Chemistry **10**

Auto-oxidation of vivianite and amorphous Fe^{2+} compounds in dried sewage sludge granules

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A main portion of dried sewage sludge is incinerated in cement industries. This important waste is reduced to ashes and its energetic content is used as an auxiliary fuel. During its storage, a significant temperature increase occurs which induces sometimes a thermal runaway.

Responsible for this sudden temperature increase is a cascade of chemical events which is initiated by the oxidation of Fe^{2+}, representing an important part of total iron ($Fe_{tot} \leq 10$ % dried mass ratio). As shown by XRD, a large fraction of this ferric iron consists of microcrystalline vivianite ($Fe_3(PO_4)_2 \cdot 8\ H_2O$) [1]. We hypothesize that the heat release of this oxidation combined with the catalytic effect of the resulting Fe^{3+} triggers the auto-oxidation of the organic matter (50 % solid content).

Dried sludge granules are excellent thermal insulators with low thermal heat capacities. This explains the occurrence of a major temperature increase within big storage tanks even in the case of a small energy release.

This work studies therefore the evolution of the $Fe^{2+}/\ Fe^{3+}$ ratio during storage of the dried sludge granules using ion liquid chromatography [2] and the decomposition of vivianite using X-ray diffraction. The kinetics of this Fe^{2+} oxidation will be important to understand the mechanism of spontaneous temperature increase.

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Analytical Chemistry **11**

Study of non-covalent complexes between human kinases and clinical inhibitors by nanoelectrospray mass spectrometry

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Protein kinases play a key role in cell regulation mechanisms and abnormal phosphorylation is often a cause or consequence of disease, like cancer and inflammation. Hence, protein kinases have become one of the most important drug targets in biomedical research. Our work is to develop a mass spectrometry based method to screen human kinase inhibitors and classify them by affinity.
Experimental parameters were optimised in order to observe non-covalent complexes in the gas phase between LCK or p38 and inhibitors. For competitive experiments [1] with p38, the protein was incubated with Bay 43-9006, as reference compound. Two other inhibitors (BIRB796, VX-745) were added subsequently at the same concentration as the reference compound. When only the complex with the tighter binding ligand is observed, the concentration of the other ligand is adjusted. A relative dissociation constant can be calculated from the ratio of the peak areas of the complexes. We obtained a relative classification for affinity in the gas phase (BIRB796>VX-745>Bay 43-9006), which is in a good agreement with data in solution [2]. The same experiments were achieved with LCK, PD173074, as reference compound, and Glivec, Bay 43-9006, Tarceva, Iressa, as competitive inhibitors. We obtained a relative classification of affinity in the gas phase (Glivec>Bay43-9006>PD173074>Iressa>Tarceva), which is also in a good agreement with data in solution [2].

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Analytical Chemistry **12**

Fast log P measurement by ultra performance liquid chromatography (UPLC)

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A suitable pharmacokinetic profile, particularly in terms of ADMET properties is required to obtain valuable drug candidates. Reversed-phase liquid chromatography (RPLC) methods represent a good alternative to the traditional shake-flask method to measure lipophilicity, a key parameter needed to define these profiles [1]. Moreover, LC has recently evolved with the development of short columns packed with small particles (sub-2 µm) working at ultra high pressures (> 400 bar), thus enabling fast and ultra-fast analysis while maintaining a good efficiency in high flow-rate conditions.

In this work, an Ultra Performance Liquid Chromatography (UPLC) system able to work up to 1000 bar was used for log P measurements. Different newly derived stationary phases packed with 1.7 µm particles were precisely characterized in terms of intermolecular forces (solvato-chromic approaches) using a well-balanced set of compounds. Isocratic as well as gradient modes were tested, and their advantages and drawbacks pointed out. The UPLC approach offered a significant increase in the throughput for the determination of lipophilicity parameters and the tested columns exhibited a very broad stability over an extended pH range ($1 < pH < 12$), suggesting that UPLC is emerging as an interesting high-throughput solution for lipophilicity measurement.

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Analytical Chemistry **13**

Lipidomic and metabolomic study by mass spectrometry in correlation with aging in *C. elegans*

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The process of aging and longevity still remains a challenging and very complex biological issue where the nematode *C. elegans* has become an important model system. It has been proved the central role of the insulin/insulin-like growth factor (IGF-1) signaling pathway in the regulation of its lifespan as well as in the flies, yeast and possibly in mammals where the vitellogenin genes *vit-2* and *vit-5* were identified as *daf*-2/*daf*-16-regulated genes. In recent studies, a possible link between longevity, lipid metabolism and reproduction has also been investigated.
Preliminary results in our group based on differential proteomic studies using wild type N2 strain and *Daf-16 C. elegans* mutant showed up-regulation of members of the vitellogenin proteins family. Consequently, we evaluated and developed different analytical strategies for profiling metabolites in the two strains by chip-based infusion approaches and high performance liquid chromatography coupled to mass spectrometry.
First, lipids from *C. elegans* were extracted by a modified Bligh-Dyer method. In a second time, identification and quantification of phospholipids and sphingolipids of crude lipid extracts were carry out by multiple precursor ion scan (MPIS) in shotgun experiment using a hybrid quadrupole time-of-flight (Qstar) and a triple quadrupole-linear ion trap mass spectrometers (Qtrap) with a nanoflow ion source. Finally, phospholipids, glycerolipids, sphingolipids, fatty acyls and *daf-2*/*daf-16*-regulated lipidic metabolites of each strain were separated by LC-MS. Data obtained were interpreted by analytical softwares to determine lipid and metabolites profiles who highlighted significant changes between wild type strain and *Daf-16* mutant in *C. elegans*

Analytical Chemistry **14**

UPLC-Q-TOF-MS/MS and CapNMR characterisation of biomarkers revealed by a metabolomic study of the wound response in *Arabidopsis thaliana*

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A metabolomic approach using LC-MS and capillary NMR (CapNMR) has been devised to search for original compounds involved in the defence mechanisms of the model plant Arabidopsis thaliana (Brassicaceae). The de novo structural determination of these compounds, mainly oxylipins, is very challenging because they are present in minute amounts within the major constitutive metabolites. Thus, an LC-MS-triggered microfractionation procedure followed by CapNMR was used for their specific isolation and subsequent characterisation at the microgram level. Specimens of A. thaliana were wounded with forceps to mimic herbivores attack and harvested after 90 minutes. To identify defence induced compounds, comparisons of extracts of control and wounded specimens were assessed by rapid UPLC-ES-TOF-MS analyses, followed by multivariate analysis. Structural information about the putative biomarkers was obtained using UPLC-Q-TOF-MS/MS analyses. However, isolation and characterisation by NMR was necessary to confirm the molecular structure of some molecules. Because of the convoluted nature of the extracts, an MS-triggered multiple step isolation procedure was performed to avoid co-elutions of targeted compounds. Firstly, enriched extracts were separated by semi-preparative HPLC using a gradient which was geometrically transferred from the UPLC gradient. To obtain maximal resolution, selected fractions were further purified on two semi-preparative columns coupled in series under optimal isocratic conditions. The latter were calculated from two precise gradients using HPLC modelling software. The fractions containing wound induced compounds were dried, directly diluted in 5 μl of deuterated solvent before analysis by CapNMR. Extensive 2D NMR experiments were obtained from 50-100 μg of the pure compounds. In this way, different polar oxylipins were efficiently isolated and characterized. The generic method developed can be applied to the investigation of any minor bioactive constituents in plants.

Analytical Chemistry **15**

Asymmetrical flow field flow fractionation–multidetection system as a tool for natural biopolymer characterization

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Natural biopolymers are extremely difficult to analyze and test because they are usually present as complex mixtures with distributions in physicochemical properties. The information obtained using non-separation based analytical tools is limited since measurements are made on the entire mixture and average values are obtained. Consequently, the property - performance relationships are also limited because it is not clear whether one or multiple components of these mixtures contribute to the macromolecule properties.
The present study explores the capabilities of the asymmetrical flow field flow fractionation (aFlFFF) with a multidetection system to characterize natural biopolymers. aFlFFF, coupled on-line to a differential refractive index and seven angle laser light scattering (LS) detectors was used to provide information on the average values and continuous distributions of molar masses, radius of gyration and hydrodynamic radius of several natural biopolymers including alginates and succinoglycan.
The hyphenation of an aFlFFF channel and a LS allows to obtain the molecular parameters in a single run without the need for calibration. However, managing such complex hyphenated system requires extensive knowledge of the mechanisms underlying each analytical technique. In the aim of a correct interpretation of the experimental results and extraction of meaningful molecular characteristics, the influence of key operating parameters, including the crossflow rate, the carrier composition and ionic strength, the accumulation wall membrane charge and the sample load on the retention and molecular characteristics was carefully evaluated.

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Analytical Chemistry **16**

Dynamic Preparation of SI-traceable Calibration Gas Mixtures

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The dynamic preparation of standard gas mixtures by means of permeation devices and dilution units is an accurate method for reactive substances like nitrogen dioxide or ammonia at very low concentrations as required for e.g. air quality monitoring [1], [2]. Microgravimetric characterisation of commercially available permeation devices, calibration of mass flows, purity analysis of the permeates and of the complementary gases are fundamental for determining the combined uncertainties of the calibration gas mixtures.
The microgravimetric apparatus, the traceability scheme for the amount of substance fraction and the mass spectrometric method are presented [3], [4]. Results are shown from the studies of the conditioning times, the temperature dependence and of the repeatability of permeation devices, the trace analysis for the purity of a NH_3 permeator and of various complementary gases [5]. An uncertainty budget with the individual sources is represented.

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Analytical Chemistry **17**

Photosensitized MALDI Matrices for Labelling Cysteinyl Peptides

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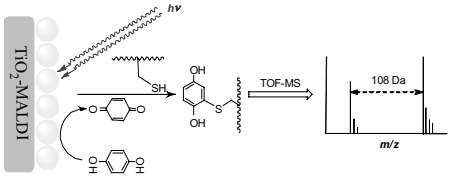
Nowadays, mass spectrometry has increasingly become one of the best methods for the analysis of complex protein mixtures. Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) has naturally found a place of choice in protein profiling [1]. In MALDI, recent advances have shown the possibility to replace organic matrices by inorganic ones based on SiO_2 or TiO_2 [2,3]. Here, we report the design of a TiO_2-based photo-reactive matrix for photo-electrochemical induced cysteine tagging during MALDI-MS. Actually, selective tagging of cysteines is an important strategy in protein profiling as it allows a better protein identification through cysteine on-line counting during MS [4].



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Analytical Chemistry **18**

Identification of TLC markers and quantification by HPLC-MS of various constituents in Noni fruit and commercial Noni-derived products

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Since the approval of Noni juice as a novel food by the EC in 2003, products derived from Noni fruit (*Morinda citrifolia*, Rubiaceae) are becoming increasingly popular as food supplements [1]. While the knowledge on constituents of Noni fruit has considerably increased over recent years, quantitative data remain scarce and the chemical composition of commercial products distributed mainly via Internet is poorly established. In the present study, HPLC and HPTLC profiles of commercial Noni juices and capsules were compared. 3-Methyl-1,3-butanediol was identified as a typical marker in Noni juices. The presence of sorbic acid (E200) was detected in one Noni juice declared as additive free.
We developed and validated an HPLC-MS method for the quantitative analysis of characteristic Noni constituents, including iridoid glucosides, scopoletin, rutin, fatty acid glucosides and anthraquinones. The separation was performed on a C-18 column with a gradient of acetonitrile in water containing 0.1% formic acid, and detection was with ESI-MS in the negative ion mode. The method was applied to the analysis of various commercial juices and capsules. Significant differences were observed between the samples. Asperulosidic acid, deacetylasperulosidic acid and rutin were present in all products analysed, but their concentrations differed greatly between the products. The fatty acid glucosides noniosides B and C were present in capsules and most juices. Scopoletin was mainly found in juices. The mutagenic anthraquinone alizarin which has been reported from roots and leaves was not detected in the investigated samples.

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Analytical Chemistry **19**

Extraction and Quantitative Analysis of Non-derivatized Glucosinolates in Plant Extracts – a Validated PLE/LC-MS Protocol

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Glucosinolates have attracted significant interest due to the chemopreventive properties of some of their transformation products [1].
Numerous protocols for the extraction and analysis of glucosinolates have been published but little effort has been devoted to optimize and validate crucial extraction parameters and sample preparation steps. Typically, the plant material is heat-pretreated and/or extracted at high temperature to inactivate myrosinase, followed by enzymatic desulfatation and analyis by reversed phase HPLC. More recently, analysis of intact glucosinolates has been attempted using ion-pair chromatography [2]. However, peak shape and separation usually were not satisfactory.
We carried out a systematic optimization and validation of a quantitative assay for the direct analysis of non-derivatized glucosinolates in *Isatis tinctoria* leaves (woad, Brassicaceae). Various parameters such as solvent composition, particle size, temperature, and number of required extraction steps were optimized using pressurized liquid extraction (PLE). We observed thermal degradation of glucosinolates at temperatures above 50°C, and loss of > 60% within 15min at 100°C, but no enzymatic degradation in the leaf samples at ambient temperature. Excellent peak shape and resolution was obtained by reversed-phase chromatography on a Phenomenex Aqua column using 10mM ammonium formate as ion pair reagent. Detection was carried out by ESIMS (negative ion mode). Analysis of cruciferous vegetables and spices such as broccoli (*Brassica oleracea* L. var. *italica*), garden cress (*Lepidium sativum* L.) and black mustard (*Sinapis nigra* L.) demonstrated the general applicability of the method.

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Analytical Chemistry **20**

Single molecule Tip-enhanced Raman Spectroscopy

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Recently, single molecule tip-enhanced resonance Raman spectroscopy of dye molecules has been claimed based on the observation of temporal spectroscopic fluctuations.[1,2] However, tip-enhanced Raman spectroscopy (TERS) of molecules not showing any resonance Raman effect is still difficult, due to the extremely small cross section of normal Raman scattering. In this work, a 2-mercaptopyridine sub-monolayer, which does not absorb visible light, was studied. Temporal intensity fluctuations of different vibrational modes were observed. This suggests that the sensitivity of our TERS setup has reached the single molecule level without the additional resonance Raman effect. Thanks to the high sensitivity of TERS, studies on biological samples become possible. We investigated different bio-molecules, including amino acids and nucleic acids deposited on Au substrates. Spectra comparable to the results by surface-enhanced Raman spectroscopy (SERS) were obtained. It provides an unprecedented opportunity to identify, monitor and analyze label free bio-systems with nanometer spatial resolution at ambient conditions.

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Analytical Chemistry **21**

Epitope mapping on bovine prion protein using chemical cross-linking and mass spectrometry

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Development of new analytical strategies for determination of epitopes, the precise region of a protein involved in the interaction with an antibody is crucial to optimize diagnostic tools. In the present study we have evaluated a new analytical approach for characterizing epitopes using the monoclonal antibody 3E7 and bovine prion protein bPrP(25-241) [1]. A combination of chemical cross-linking and mass spectrometry was applied [2]. Cross-linking reactions were conducted with isotope-labeled, amine reactive cross-linkers, disuccinimidyl suberate (DSS-d0/d12) and disuccinimidyl glutarate (DSG-d0/d6). After cross-linking, the covalently bound immuno-complex was analyzed directly using a Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometer equipped with an Ion Conversion Dynode (ICD) high-mass detector. The sample containing a high proportion of the specific cross-linked complex was digested by chymotrypsin and the peptides obtained were analyzed by nano-LC-ESI-FTICR mass-spectrometry. As a result, a complete fading of the peak intensities corresponding to the peptides representing the epitope was observed. The amino acids 150-160 are involved in the binding to the antibody. Our results showed excellent agreement with pepscan data. The epitope of 3E7 on bPrP(25-241) was determined, with only a low amount of sample (200 picomoles) needed.

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Analytical Chemistry **22**

Cationic terpolymers composed of bis1,3(N,N,N-trimethylammonium)2propylmethacrylate dichloride, acryloyloxyethyltrimethylammonium chloride and acrylamide: Analysis of composition and electrochemical behavior

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Charged macromolecules, polyelectrolytes (PEL) are used for a broad variety of applications. Frequently, copolymers or even terpolymers have better application performance than homopolymers. In such cases the product analysis and product quality control become a challenge, in particular, if the chemical structure of the monomer units forming the polymer chain is very similar and the polymers have high molar masses.
This contribution presents a procedure to analyze quantitatively the composition of terpolymers composed of bis1,3(N,N,N-trimethylammonium) 2propylmethacrylate dichloride (dipole M), acryloyloxyethyltrimethylammonium chloride (Q9), and acrylamide combining FTIR and potentiometric titration. The procedure is based on previous calibration with appropriate copolymers. Comparing the initial monomer feed composition and the product composition allows conclusions concerning the monomer reactivity. Counterion activity measurements reveal the influence of the solution viscosity on the ion mobility. Overall, FTIR present a practically useful simple method to analyse the composition of these terpolymers and to control the polymer production.

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Analytical Chemistry **23**

Analysis of endogenous metabolites of a single yeast cell lysate

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Metabolomics is a useful tool for high throughput analysis of the biochemical phenotype of a cell. Conventional analytical methods provide data that are averaged over many cells, while even cells in monoclonal cultures display strong variations on all levels. Thus a single cell approach to metabolomics is essential to create accurate models that consider the stochastic nature of many cellular events.
Single cell analysis is difficult due to the minute amounts of samples in each cell. We are addressing this challenge by coupling a microfluidic cell processing and sample preparation step to a novel matrix assisted laser desorption/ionization – mass spectrometry (MALDI-MS) method. We demonstrate that the combination of piezoelectrically-driven nanoliter spotting with thin layer preparation meets the needs of single cell analysis in terms of sample consumption and limit of detection. The method is also tested to determine metabolite levels in yeast cells extracts. The spotting of the sample was conducted by depositing a variable number of 500pL drops. Spotting of the solution premixed with matrix was compared with the spotting onto a thin layer of matrix. Both methods showed a limit of detection (LOD) in the attomole range, at least two orders of magnitude below the one obtained with classic pipette spotting. Even spots with a sample volume as minute as 500pL gave a detectable signals of metabolites reaching for some of them a LOD (90amol for adenosine 5'-triphosphate) comparable with the content in a single yeast cell. The same methods were then applied to yeast cell extract. Finally the spectrum of the cell extract corresponding to a single cell was obtained by spotting 500pL of diluted yeast cell extract.

Analytical Chemistry **24**

CZE and MEEKC coupled with APPI/MS for pharmaceutical applications

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The electrospray ionization (ESI) source is currently the method of choice for CE-MS because of its sensitivity, versatility and ease of implementation. Nevertheless, ESI presents some inherent drawbacks mainly related to large signal suppression which can occur with complex matrices or non volatile additives. Therefore, partial filling techniques have to be implemented for chiral, micellar (MEKC) or micro emulsion (MEEKC) analyses to avoid background noise and source contamination. The atmospheric pressure photoionization (APPI) source is described to be less sensitive to matrix effect and to the presence of additives and broadens the range of ionizable analytes to non-polar compounds. This ionization source is similar to APCI with the corona needle replaced by a krypton discharge lamp. In this configuration, the sample is vaporized prior to ionization and non-volatile salts can be therefore eliminated during this step.
In this study, numerous CE-APPI/MS parameters were optimized thanks to an experimental design strategy. Generic conditions adapted for polar as well as non-polar compounds were found and limits of detection were determined in CZE-APPI/MS for a pharmaceutical mixture and compared to conventional CZE-ESI/MS. Furthermore, possibilities to enhance selectivity by the direct coupling of MEEKC with APPI/MS were highlighted for the complex separation of ionized and neutral compounds in positive and negative APPI modes. System stability as well as method sensitivity and efficiency were emphasized. Application to screening analysis of doping substances (such as β-blockers, steroids, diuretics…) was finally implemented.

Analytical Chemistry **25**

CE-LIF analysis of pharmaceutical compounds in biological fluids: evaluation of various derivatization modes

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Analysis of pharmaceutical drugs and metabolites at low concentration levels in biological matrices requires sensitive methods since many drugs show a high volume of distribution. In order to reach detection limits in the ng/mL range, laser-induced fluorescence (LIF) was hyphenated to CE to ensure sensitivity and selectivity. However, few compounds possess a strong and native fluorescence, a derivatization procedure is therefore usually mandatory.
In this study, three derivatization procedures were assessed for CE-LIF analysis of pharmaceutical compounds in plasma (such as myorelaxants, stimulants, β-blockers): liquid phase pre-capillary derivatization, solid phase pre-capillary derivatization and on-capillary derivatization. Various laser sources were evaluated (442 nm gas laser *vs*. 410 nm diode laser), as well as derivatization tags (naphtalene-2,3-dicarboxaldehyde *vs*. fluorescamine) and electrophoretic modes (CZE *vs*. MEKC). Limits of detection down to 5 ng/mL in plasma were reached which enhanced sensitivity compared to conventional CE-UV. Finally, another CE-LIF method, without fluorescent tagging thanks to a 266 nm diode laser, was developed and compared to other approaches.

Analytical Chemistry **26**

Nanoscale molecular analysis and chemical imaging via atmospheric pressure near field laser ablation mass spectrometry

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We present an instrument that achieves chemical analysis of samples at atmospheric pressure with nanoscale spatial resolution by coupling mass spectrometry to laser ablation via scanning near field optical microscopy (SNOM). No other method available today offers this unique analytical capability, since classical techniques for the characterization of materials on the nanoscale, such as standard AFM, STM and electron microscopy, yield little or no chemical information at all, and most traditional chemical analysis methods are far from having the required spatial resolution.

As opposed to a previous SNOM-MS design by Kossakovski [1] which required the sample to be in high vacuum, our setup allows the analysis to be performed at ambient conditions. In previous work by our group [2], we demonstrated the feasibility of nanoscale-MS at atmospheric pressure, but analysis at that time was limited in mass spectral performance. We have now developed a combination of an optimized ion trap and time-of-flight MS [3] which allows for accumulation of the sampled material and which allows for simultaneous detection of a wide range of mass-to-charge ratios.

A characterization of the instrument performance with different samples will be presented. The near field ablation craters with their corresponding mass spectrometry signatures will be demonstrated.

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Analytical Chemistry **27**

Towards Rapid Nanoscale Chemical Analysis using Tip-Enhanced Raman Spectroscopy with Ag-Coated Dielectric Tips

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Since the introduction of tip-enhanced Raman spectroscopy (TERS) in 2000, intense research efforts have been made to push the frontier of this technique towards reproducible nanometer scale chemical analysis. The ability to perform such measurements is of great importance, e.g., for the understanding of catalytic processes and for the fabrication and quality control of molecular electronics.

In this work, the influence of dielectric substrates on the Raman scattering activities of Ag overlayers has been investigated. Materials with low refractive indices such as SiO_2, SiO_x and AlF_3 have been found suitable as supporting platforms for Ag films at 488 nm illumination to give strong surface-enhanced Raman scattering for dye molecules. This finding is then extended to TERS. Huge observed enhancements of 70-80 ×, corresponding to net enhancements of $>10^4$, have been achieved for brilliant cresyl blue test analyte using Ag-coated tips made from or pre-coated with low refractive index materials. The yield of tips giving significant enhancement to the Raman signals is found to be close to 100%. These findings are crucial steps towards the use of TERS as a robust technique for rapid chemical imaging with nanometer spatial resolution.

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Analytical Chemistry **28**

Laser Ablation-ICP-MS: Quantification of femtosecond laser ablation generated aerosols using solution nebulization for calibration

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The use of nebulized liquid standards to quantify LA-aerosols in the ICP-MS has first been proposed by Thompson et al [1] and recently O'Connor et al [2] show the usability of this approach for the majority of the elements for different matrices.
Since femtosecond laser systems have shown there ability to produce aerosols which are stoichiometric representative for the sample [3], in this study a Ti:Sapphire fs-laser system operated at its 3rd harmonic (265nm) was used to ablate conducting (brass) and nonconducting (glass) materials. For calibration, liquid standards are nebulized and applied directly or via a membrane-desolvatisation unit to the plasma. The desolvatisation step was implemented to reduce spectral interferences caused by higher oxide building rates through the addition of water to the plasma. During ablation a blank solution was nebulized to ensure that plasma conditions remain as constant as possible. To compensate for the different mass flows from the nebulizer and the laser ablation unit, Calcium and Copper were used as internal standards for glass and brass samples respectively.
Most of the determined elements show a deviation of in the range of 1% to 10% against the certified reference values regardless if it concerns main or trace elements. However, especially in the glass samples some elements like Zinc and Selenium show deviations up to 50%.

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Analytical Chemistry **29**

UPLC at high temperature as a highly efficient technique for the separation of complex pharmaceutical mixtures.

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Pharmaceutical industry has always been interested by highly efficient analytical techniques, to solve very complex pharmaceutical cocktails or in drug impurity profiling (i.e. to deal with a high number of impurities). Additionally, with the development of genomics, proteomics and more recently metabolomics, there is a need for analytical procedures able to yield high resolution in acceptable analysis time.
Among the existing solutions to improve efficiency in liquid chromatography (LC), the use of UPLC (Ultra Performance Liquid Chromatography) in high temperature mobile phase conditions (HT-UPLC) represents an adequate approach to improve the efficiency while maintaining an acceptable analysis time. Indeed, this approach is compatible with long columns packed with sub-2μm particles, because the mobile phase viscosity decreases with temperature.
This procedure was investigated in isocratic as well as gradient mode. In order to obtain high performance in HT-UPLC, a compromise has to be found between column length, working mobile phase flow rate, efficiency and generated back pressure. This work presents some advantages and drawbacks of HT-UPLC for high resolution separations.
With this approach, it is possible to reach ca. 100'000 plates in isocratic mode, with a suitable analysis time (less than 1 hour), using a 450 mm column length packed with 1.7μm at 90°C and 1000 bar. Finally, HT-UPLC was applied for the separation of complex mixtures (35 pharmaceutical compounds and 35 doping agents) in gradient mode, for column length ranging from 30 to 450 mm. Some limitations have been established in both modes, depending on the used column length (i.e. impossibility to work at optimal flow rate, potential compounds degradation for very long separations) and some rules are given to select the appropriate column length.

Analytical Chemistry **30**

Characterization of Monoclonal Antibodies: Surface Plasmon Resonance or High-mass MALDI Mass Spectrometry?

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The characteristics of antibody-antigen interaction such as the nature of the epitope, the ability of the antigen to bind two antibodies simultaneously and the kinetic and dissociation constants are usually determined using Surface Plasmon Resonance (SPR). This method requires that interacting partners be separated: one is immobilized while the other is flowing across the surface.
The development of a new protocol using cross-linking chemistry associated with high-mass mass spectrometry using matrix assisted laser desoprtion ionization (MALDI MS) allows characterization of monoclonal antibodies directly in solution. Samples are stabilized with cross-linking chemistry prior the analysis with a high mass detector system (CovalX).
We characterized the binding between the monoclonal antibody 1E5 and the bovine prion protein (bPrP) (sequence of the binding site, multibinding studies and kinetic measurements) comparing high-mass MALDI mass spectrometry and SPR. Multibinding experiments were performed with a second antibody against bPrP, and epitope mapping was achieved by a competition assay with competing peptides.

Analytical Chemistry **31**

**Your 1D ^{13}C NMR experiments last too long?
Try our new 2D long-range experiment with high-resolution in the carbon dimension!**

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Most chemists have no time or availability to apply some of the most powerful NMR technique even when they provide significant resolution enhancement or reduction in experimental time.[1] They need ready-to-used experiments. We have developed such a new 2D long-range experiment providing the high resolution of 1D carbon spectra and the high-sensitivity of proton detected 2D experiments.
In standard 2D long-range experiments, the accuracy of the carbon chemical shifts is usually not much below one ppm making it impossible to resolve close carbons. We developed a complementary 2D experiment providing 20-fold resolution improvement in the carbon dimension. In the resulting spectra, nearly degenerated signals can be resolved and the values of the carbon chemical shifts can to be extended to two digits after the period. The new complementary experiment requires nearly the same experimental time as the standard long-range experiment.

Application to a small amount of a mixture of two naturally occurring natural products is used as a demonstration.

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Analytical Chemistry **32**

Getting to the Bottom of Chemical Cross-linking:
Reactivities of Different Amino Acids

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Chemical cross-linking, in combination with Matrix Assisted Laser Desorption/Ionization (MALDI) mass spectrometry, has emerged as a powerful tool for the identification of noncovalent inter- or intramolecular interactions in proteins. Despite the large number of applications, only a few studies concerning the reactivity and selectivity of cross-linkers toward certain amino acids have been reported [1 - 3]. In order to understand the specificity and reactivity of different cross-linkers, systematic studies were peformed on small peptides (up to 10 amino acids) with the minimum number of reactive groups. NHS esters described usually as amine-reactive, but sometimes showing reactivity towards hydroxyl containing amino acids, were applied to lysine, tyrosine and/or serine containing peptides. MALDI-MS allows a rapid identification of the number of amino acids modified by the cross-linker. The so-called decoration of the amino acids was highly dependent on the reaction conditions. The hydroxyl containing amino acids serine and tyrosine appeared much more difficult to modify than lysine under comparable conditions. The formation of intermolecular links between two peptide molecules was observed only in exceptional cases. This demonstrates that chemical cross-linking of biological molecules is mainly observed for specific protein/peptide interactions.

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Analytical Chemistry **33**

Hydroxylated Metabolites of Hexachlorocyclohexanes: Bacterial Formation and Stereochemical Configurations

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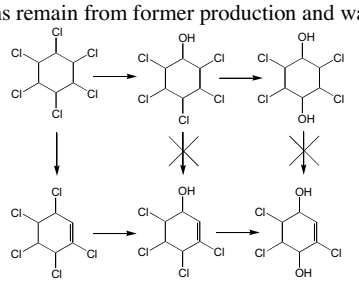
Although the use of hexachlorocyclohexane (HCH), one of the most popular insecticides after the Second World War, has been discontinued in many countries, problems remain from former production and waste sites.



We isolated and characterized hydroxylated metabolites from HCH incubation experiments with the common soil microorganism *Sphingobium indicum* B90A [1]. The regio- and stereo-selectivity of the hydroxylation mechanism is discussed. Several of the metabolites were detected in groundwater from a former HCH production site in Switzerland.

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Analytical Chemistry **34**

Roles of dynamic metal speciation and membrane permeability in the metal flux at permeation liquid membranes

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In natural waters, trace metals are present in a large number of different forms. In order to understand their bioavailability for microoganisms, the study of the role of different metal species on the metal flux related to biouptake is a key issue. Permeation Liquid Membrane (PLM) is a promising technique for trace metal dynamic speciation, because it is a simple technique, its sensitivity is high due to its preconcentration capability and the processes of metal transport through PLM are similar to those occurring in biological membranes. In PLM the metal ion is transported through a lipophilic liquid membrane, by complexation with a lipophilic ligand which serves as carrier. The important parameters which influence the metal flux are the diffusion layer and membranes thicknesses, the diffusion coefficients of the metal ion and its complexes, the chemical association and dissociation rate constants of the metal complexes in solution, the partition coefficient of metal ion between the solution and the membrane, and the nature and concentration of the carrier. A general model relating these parameters to the steady-state flux of metal through the PLM, has been developed. It is applicable to complexes with any degree of lability (from fully labile to inert). This model has been validated experimentally with complexes with varying degree of lability, in particular: Pb-NTA, Pb-TMDTA, Pb-Diglycolate, Cu-Diglycolate and Cu- N-(2-Carboxyphenyl)glycine complexes. It enables to predict the role of the different types of natural complexes on the bioavailability of metals for organisms and it serves as a theoretical basis for PLM sensors which ebnable to determine the ecotoxicological roles of metals in aquatic systems.

Analytical Chemistry **35**

Investigation of aerosol transport parameters on signal response in LA-ICP-MS

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The transport process of laser generated aerosols influences the detection capabilities of laser ablation microanalysis. Therefore, beside important factors like ablation cell volume, cell geometry, gas flow pattern [1, 2], the volume (length) of the transport tube is another relevant factor, which needs to be investigated.

The aim of this study was to investigate the aerosol manipulation in LA-ICP-MS. The signal intensity changes of various elements and the matrix-related changes were determined and compared for different transport systems. For these measurements variable mass loads were introduced to the ICP [3]. The experiments were carried out using ns and fs laser ablation systems and NIST 610, brass standard reference materials as samples. The influential parameters related to the transport system will be discussed in this presentation.

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Analytical Chemistry **36**

Determination of gas-phase basicity of peptides and proteins at atmospheric pressure by ESSI-MS

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ESI is a powerful method to analyse proteins and non-covalent complexes in the gas phase. The ionisation process leads to the formation of multiply charged ions for large compounds. The ionisation mechanisms still remain unclear, although there is some evidence of the validity of the Charged Residue Model and for the control by apparent gas-phase basicities (GB_{app}).
In this work, we present a fast and innovative methodology, based on ESSI-MS, to determine GB_{app}s of peptides and proteins at atmospheric pressure. Vapor of volatile reference bases was introduced to react with the protein ions in the atmospheric pressure region, before the MS inlet. The vapor pressure was close to the saturation pressure, ensuring a high collision rate with the protein ions and efficient deprotonation reactions. The proof of principle was made using bradykinin derivatives, substance P and insulin chain B. We obtained values in excellent agreement with the GB_{app} values obtained at low pressure (kinetic or bracketing methods).
These experiments were extended to model proteins (unbiquitin, lysozyme and cytochrome c) in denaturing and non-denaturing buffer. The results were compared to the GB_{app} calculated with an electrostatic model and confirm that GB_{app} control of the protein ionization in the gas phase. Moreover, some evidence of the presence of ion pairing between ionized basic and acidic sites are given by these measurements.
ESSI-MS offers the unique possibility to measure GB_{app} of peptides and proteins at atmospheric pressure with good sensitivity (for concentrations less than 10 μM in denaturing or non-denaturing buffer), very good accuracy (less than 2%) and in a short time (less than 30 minutes to screen up to 23 volatile bases).

Analytical Chemistry **37**

Mass Load Induced Matrix Effects in LA-ICP-MS: A Further Insight

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Laser ablation (LA) is nowadays broadly used as a sample introduction method for inductively coupled plasma mass spectrometry (ICPMS) due to its easy sample preparation, fast handling and its potential for high spatial resolution. However, amount and composition of laser generated aerosols strongly depend on the matrix, especially on the ablation characteristics of the sample. Differently sized and composed particles can then cause fractionation by incomplete vaporization, atomization and ionization of large particles in the plasma[1] and make the use of closely matched ablation conditions and calibration standards crucial. This was emphasized by Kroslakova[2] who found a matrix effect induced by the mass load of the plasma: higher plasma loads result in a subsequent loss of especially the volatile elements relative to Calcium, a lowering of the plasma load leads to the opposite effect.
In this study, these effects were further evaluated. The focus of the mass load studies was extended to different matrices (e. g. the less absorbing NIST612, zircon) to study the relation of the sample matrix and its subsequent ablation behavior with the degree of mass load bias. Additionally, the influence of water addition to the ICP was studied, since wet plasma conditions have been found to provide more stable ionization conditions[3]. Mass load experiments were therefore carried out with a small amount of water added to the laser aerosol by a PFA microflow nebulizer and will be discussed.

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[2] I. Kroslakova, D. Günther, *J. Anal. Atom. Spectrom.,* **2007**, 22, 51-62.
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Analytical Chemistry **38**

Levels of oxidative stress biomarkers in workers exposed to Diesel Exhaust Particulate

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The increase of exposure to PM_{10} and $PM_{2.5}$ (particulate matter with aerodynamic diameter smaller than 10 µm and 2.5 µm, respectively) has been found to be associated with a range of adverse health effects. Surface characteristics (chemical reactivity, surface area) are of prime importance to understand the mechanisms which lead to harmful effects. A hypothetical mechanism to explain these adverse effects of particulate matter is the ability of some components (organics, metal ions) adsorbed on these particles to generate reactive oxygen species (ROS), and thereby to cause oxidative stress in biological systems. ROS can attack almost any cellular structure, leading to the formation of a wide variety of degradation products which can be used as a biomarker of oxidative stress.

The aim of the present research project is to demonstrate an association between the exposure to Diesel Exhaust Particulate (DEP) and the oxidative stress status. For that purpose, a survey is conducted in real occupational situations where workers are exposed to DEP (bus depots).

Analytical methods allowing the determination of several biomarkers of oxidative stress in urine or serum of volunteers have been validated. Results about biomarkers levels determined in such matrix will be presented and discussed for their applicability and in relation to the particulate exposure variables.

Analytical Chemistry **39**

Ultra-fast quantitation of saquinavir in human plasma by MALDI-SRM

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Quantitation of low molecular weight compounds (LMWC) in biological samples by low laser frequency matrix-assisted laser/desorption time-of-flight mass spectrometers (MALDI-TOF) remains a challenging task. The hyphenation of MALDI with triple quadrupole mass spectrometers (MALDI-QqQ) has been shown to open new opportunities in the quantitation of LMWC [1].
We illustrate herein its potential in the selected reaction monitoring mode (MALDI-SRM) using a MDS Sciex MALDI-QqQ prototype equipped with a high repetition (1 KHz) laser for clinical chemistry purposes. Saquinavir, an anti-HIV drug largely employed in AIDS multitherapies, is taken for example.
Three important aspects are highlighted:
1. The dramatic throughput improvement allows ultra-fast quantitation (less than 10 seconds per sample), over a range of concentrations (5 to 10'000 ng/ml) meeting those covered in clinical trials.
2. The spotting process has been automated with a processing station (Shimadzu Xcise), customized to perform electrodeposition.
3. Specific experimental design has been employed to tackle the reproduciblity issues of MALDI and are discussed: a. the use of an internal standard, b. the rastering mode of the laser beam and c. repeating the spotting of each sample.

[1] P. Kovarik, C. Grivet, E. Bourgogne, G. Hopfgartner, *Rapid Commun. Mass Spectrom.*, **2007**, *21*, 911-919

Analytical Chemistry **40**

LA-ICP-MS - a versatile analytical technique in material science

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Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a fast and versatile analytical technique dedicated for the analysis of conducting and non-conducting solid materials. In recent years, laser ablation as sampling technique coupled to ICP-MS was frequently applied for trace element quantification in solid materials of high purity [1]. One major advantage is that it does not require time consuming sample preparation and digestion. Therefore, this sampling technique has promising potential for quality control of industrial samples.
In this study GeSbTe phase change materials were characterized for stoichiometry and surface impurities in chromium were determined after different cleaning procedures. All measurements were carried out using a 193 nm ArF Excimer laser system (GeolasPro, Coherent Lambda Physik, Göttingen, Germany) equipped with a computer-controlled stage and a petrographic microscope. The applied inductively coupled plasma mass spectrometer was an Agilent 7500ce (Agilent Technologies, Tokyo, Japan).
Four different ablation procedures (single pulse, drilling-mode, raster-mode, and matrix ablation) were compared and discussed in detail concerning their capabilities and limitations. Using single pulse ablation a depth resolution of 300-400 nm per pulse was achieved for depth profiles in Cr. Due to the uniform particle size distribution and the constant mass load into the plasma over the entire ablation period, raster mode showed highest precision in terms of stoichiometry determinations on the phase change materials using matrix-matched in-house reference samples for calibration. Furthermore the sample homogeneity was investigated comparing all four ablation techniques.

[1] J. S. Becker and H. J. Dietze, Int. J. Mass Spectrom., **2003**, *228*, 127

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Analytical Chemistry **41**

Sensor Arrays for the Analysis of Sugars in Aqueous Solution

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Fluorescence sensors for sugars have received enormous interest in recent years. Most efforts have focused on the development of sensors with a highly selectivity for a particular sugar. This is generally accomplished with the help of synthetic receptors, which display a high specificity. An interesting alternative is the utilization of a sensor array technology. In a sensor array, several non-specific sensors are combined and the analyte is then identified with pattern recognition tools. This technique has successfully been applied for different analytical problems [1], but the utilization of sensor arrays for sugars is virtually unexplored [2]. We describe a sensor array, which is based on the reversible coupling of fluorescent hydrazides with the aldehyde group of reducing sugars.

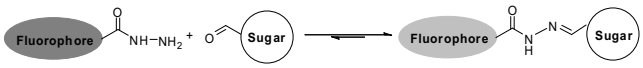

Fig.1.: Schematic representation of a fluorescence sensor for sugars

Discrimination is achieved by exploitation of differences in fluorescence emission intensities which depend on the nature of the dye-sugar derivate and the reaction equilibria in solution.

[1] K. J. Albert, N. S. Lewis, C. L. Schauer, G. A. Sotzing, S. E. Stitzel, T.P. Vaid, D. R. Walt, *Chem. Rev*. **2000**, 100, 2595.
[2] J. W. Lee, J.-S. Lee, Y.-T. Chang, *Angew. Chem. Int. Ed.* **2006**, 45, 6485.

Analytical Chemistry **42**

Investigation of *Hypericum* species by LC/MS

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A characteristic of plant species from the genus *Hypericum* (Hypericaceae) is the presence of pigments belonging to the class of naphthodianthrones. These plants have many traditional uses and, notably, *Hypericum perforatum* is employed for the treatment of mild depression. Several studies deal with the activities of the numerous constituents of the genus or compare different *Hypericum* species [1, 2]. The genus is also reputed for cases of poisoning in cattle (hypericism) which also have their origin in the presence of these compounds. More recently, the naphthodianthrones have assumed importance for the photodynamic therapy of cancer.
In order to determine the relative contents of hypericin and pseudohypericin in these plants, extraction of several species of St.-John's wort was performed by different procedures in order to optimize the yield of the active constituents.
HPLC-UV/DAD and HPLC-MS methods were then developed for the analysis of naphthodianthrones in the plants. It was found that *Hypericum calycinum* L. does not contain this class of compounds.

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Medicinal Chemistry **43**

Thermodynamic and Kinetic Considerations of the Binding Process of MAG-Antagonists

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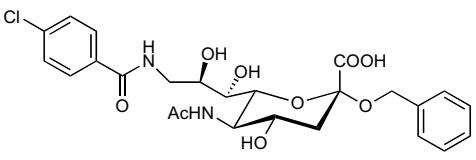
The injured adult mammalian central nervous system is an inhibitory environment for axon regeneration due to specific inhibitory proteins. The myelin-associated glycoprotein (MAG) [1] was identified as one of these neurite outgrowth inhibitory proteins [2]. It belongs to the siglec family (sialic-acid binding immunoglobulin-like lectin). In earlier studies, we identified the lead structure **1** [3], which was further optimized yielding antagonists with nM affinities.



1

The kinetic and thermodynamic properties of these high affinity ligands were elucidated by Biacore studies. In addition, the binding mode was examined through STD NMR experiments and docking studies.

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Medicinal Chemistry **44**

Novel guanidine-type 5-HT$_{5A}$ receptor antagonists

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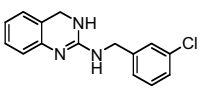
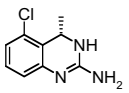
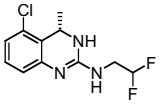


1
K_i (5-HT$_{5A}$) = 160 nM
screening hit



2
K_i (5-HT$_{5A}$) = 2 nM
brain/plasma ~ 0.2



3
K_i (5-HT$_{5A}$) = 3 nM
brain/plasma ~ 4

The expression of the 5-HT$_{5A}$ receptor in the limbic brain areas suggests a potential role in the modulation of psychiatric diseases.[1] However until recently, no selective 5-HT$_{5A}$ receptor ligands were available to study its pharmacology in detail. We screened the Roche compound library to identify selective antagonists for this target, and found several guanidines such as **1** among the most selective compounds. A systematic exploration of small substituents (Cl, Me, MeO, F) around the core structure led to **2** with potent 5-HT$_{5A}$ antagonistic affinity *in vitro*, and improved selectivity, apart from 5-HT$_7$. Compound **2** had good PK properties, however a low brain-plasma ratio. The brain penetration was improved by the introduction of electron-withdrawing substituents, which afforded a compound with increased lipophilicity, and reduced basicity, **3**. The series refinement and structure activity relationship elucidated in progressing from the initial hit **1** to lead compound **3** will be further described in the presentation.

[1] Thomas, D. R. *Pharmacol. Ther.* **2006**, *111*(3), 707-714.