ASAC

AUSTRIAN SOCIETY OF ANALYTICAL CHEMISTRY

Young Analytical Chemists Forum 2022

12th-13th May

Book of Abstracts

Thursday 12th May

08:30 - 09:30 Registration
09:30 - 09:45 Welcome
10:00 - 11:15 Session 1
11:15 - 11:45 *Coffee Break*
11:45 - 13:00 Session 2
13:00 - 14:00 *Lunch Break*
14:00 - 15:00 Session 3
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18:00 Departure Conference Dinner
18:30 Conference Dinner

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12:15 – 13:30 *Lunch Break*
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KNAUER

Session 1

12th May, 09:45 – 11:15, Polymer and Material Characterization

	09:45 – 10:00	**Welcome / Introducing ASAC** Rudolf Krska
1	10:00 – 10:15	**JAF-Award Lecture: Comparison of the Functional Barrier Properties of Chitosan Acetate Films with Conventionally Applied Polymers** Andrea Hochegger, Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology
2	10:15 - 10:30	**A LIBS based classification approach for encapsulation materials to ensure the reliability of electronic devices** Veronika Zeller, TU Wien, Institute of Chemical Technologies and Analytics
3	10:30 – 10:45	**Chloride Sensitive Dyes for Concrete Imaging** Karl Leonard Sterz, Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology
4	10:45 – 11:00	**Analysis of Poly- and Perfluoroalkyl Substances in Paper and Board Food Contact Materials** Milica Jovanovic, Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology
5	11:00 – 11:15	**Laser Ablation combined with Electron Ionization-MS and ICP-OES for advanced polymer characterization** Laura Kronlachner, TU Wien, Institute of Chemical Technologies and Analytics

Comparison of the Functional Barrier Properties of Chitosan Acetate Films with Conventionally Applied Polymers

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Paper and board are one of the most important packaging materials. The current demand towards sustainable and environmental-friendly packaging materials further increases the use of recycled materials in particular. The main disadvantage of recycled materials is the risk of undesired carry-over of substances; due to the mixing of fibre materials of different intended use and quality, a huge pool of possible contaminates is generated in the recycling process. Contaminates are not quantitatively removed during the recycling process, accumulated in the final product and can than migrate into packed good during transportation or storage. For example, the contamination of food with mineral oil hydrocarbons (MOSH & MOAH) is a huge issue.
At the moment the only fast and practical solution to prevent migration seems to be the application of functional barriers in the packaging. Most of the recently applied barriers are synthetic, often have only moderate barrier functionalities and in addition reduce the environmentally-friendly character of cellulose-based materials. Those include e.g. barriers made of PE, PP, aluminium or highly-complex multilayer materials.
Against this background, this work evaluated bio-based polymers in terms of their functional barrier properties. A two-sided migration experiment was developed to comprehensively test the barrier properties of the polymers. The method allowed not only to test the intrinsic migration of the films and the permeation through them, but also to simulate real packaging situations by using a recycled paper as donor for MOH. Due to the fact that transport phenomena are mainly driven by (gas phase) migration, gas chromatographic based techniques are ideal combination to complete the testing procedures. Due to the big demand to get detailed information on the composition of complex samples and the identification of unknown substances in the untargeted approach, a combination of various GC methods was used to qualify and quantify the migrated substances: GC-FID was used to determine the overall migration, GC-MS for specific migration, online coupled HPLC-GC-FID for the migration of mineral oil hydrocarbons (MOSH/MOAH) and finally, comprehensive GC×GC-MS was used to get a deeper inside into the complex composition of the recycled materials and the migrated fractions.
Chitosan was found to be among the best performing bio-based polymers. This talk presents the developed testing procedure and the resluts of a lab-made chitosan acetate film, compared with conventionally produced polymer films.

Keywords: functional barrier; migration; biopolymer; mineral oil hydrocarbons; gas chromatography;

A LIBS based classification approach for encapsulation materials to ensure the reliability of electronic devices

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Nowadays, we use and interact with electronic devices on a daily basis. Electronic devices are not only used as consumer electronics but are also omnipresent in crucial applications where failure may have grave consequences. This includes applications in any form of transportation (e.g., cars, trains, airplanes) or in the medical field. Therefore, the reliability of electronic devices is of major concern for chip manufacturers. The main part of an electronic device protecting the sensitive miniaturized individual components from the environment is the encapsulation material. This material is usually a composite material consisting of an organic polymer with inorganic filler particles and a wide range of (in)organic additives. Encapsulation materials are typically obtained from external suppliers providing a wide range of products with different compositions and properties. The selection of the appropriate encapsulation material for specific applications is crucial to ensure the required reliability.

In this work, we investigate the capabilities of Laser-Induced Breakdown Spectroscopy (LIBS) to classify encapsulation materials used in the semiconductor industry. LIBS is a promising technique for this task, providing fast simultaneous multi-element analysis with additional polymer-specific information and practically no sample preparation. Using a selection of observed atomic emission signals originating from inorganic and organic components present and molecular emission signals originating from the polymer, we investigate if discrimination and classification of more than 20 different encapsulation materials is possible. Next, we evaluate if we can find patterns in the dataset, enabling the classification of four different suppliers. Therefore, we use various multivariate data evaluation strategies, including Principal Component Analysis (PCA) and Random Decision Forests (RDF). The developed classification models enable an easy and fast way to check whether the supplier provided the requested encapsulation material, which is crucial to assure the required reliability. Additionally, the model may be extended to build a database for estimating the reliability of new materials.

Keywords: LIBS; multivariate statistics; classification; electronic devices

Chloride Sensitive Dyes for Concrete Imaging

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Concrete is a universally used construction material for, among others, its flexibility, durability and general chemical resistance. Despite its usefulness, concrete materials can be prone to degradation through distinct sources, like carbon dioxide diffusion and sodium chloride ingress. Previously, a pH-sensitive sensor material has been applied in a fluorescence time-domain dual lifetime reference (*t*-DLR) setup to visualize the carbonation front in concrete samples, providing a valuable addition to the toolbox of concrete analysis.[1]

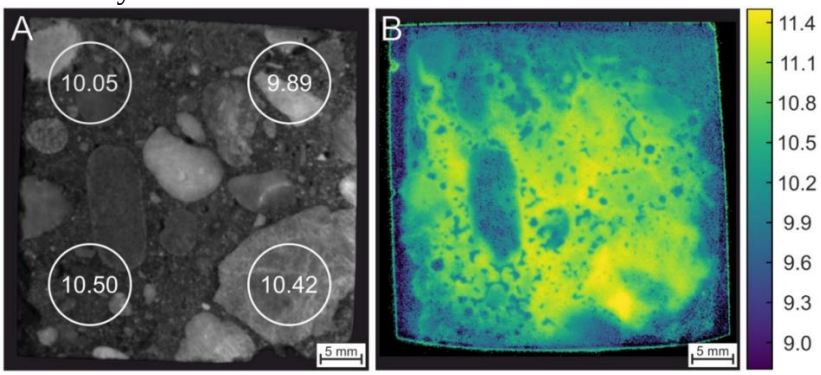

Figure 1: (left) Image of concrete surface with distinctly measured pH-values through a flat surface electrode; (right) Image of concrete surface through t-DLR measurement[1]

In the present work, the chloride sensitive dye 10,10'-Dimethyl[9,9'-biacridine]-10,10'-diium dinitrate (Lucigenin) was applied to yield a t-DLR setup for chloride measurement. Furthermore, novel sensor materials were developed, based on *N*,*N'*-Dimethylperopyrenium, Lucigenin and *N*,*N'*-Dimethyl-Diaza-Dibenzo-Perylenium compounds.

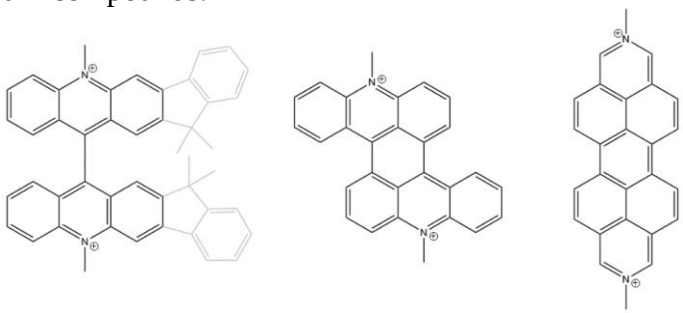

Figure 2: *Chloride Sensitive Structures for Potential Sensor Application*

Each of these compounds exhibits chloride sensitivity yet they require different solutions for successful integration into sensor foils.

Keywords: Chloride, Sensor, *t*-DLR, *f*-DLR, Fluorescence

References:

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Analysis of Poly- and Perfluoroalkyl Substances in Paper and Board Food Contact Materials

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Poly- and perfluoroalkyl substances (PFAS) is a large heterogenous group of chemicals consisting of several hundred different structures. Due to their stability and water/lipid repellent properties they are used in various applications, like production of non-stick cookware, stain resistant products or food packaging materials. However, because of their persistent and bio accumulative nature, these substances are omnipresent in the environment.

Until today, more than 5000 different PFAS are present on the market, with different physical and chemical properties. Furthermore, there are numerous precursors, many of which with unknown structures, making their analytical determination quite challenging.

Recently some PFAS have begun being regulated or phased out. Only few of the substances have been risk assessed by the European Food Safety Authority (EFSA) and Environmental Protection Agency (EPA). Currently, there are various regulatory initiatives that specify acceptable limits of some PFAS, however they have been imposed mainly for environmental matrices.

The broad distribution of PFAS, as well as concerns about their toxicity, lead to a need for continuing development of effective analytical techniques and methods to analyse them in food contact materials.

In this work, the targeted approach for detecting and quantifying 24 PFAS commonly found in paper and board matrices using high-performance liquid chromatography coupled with triple quadrupole mass spectrometry is shown. Additionally, we present the possible challenges associated to PFAS analysis, and potential solutions.

The model that we present here could lead to an establishment of simple and efficient method for quantification of PFAS that could be used for quick and easy monitoring of PFAS in paper based FCM that are currently available in the market.

Keywords: Food contact materials, PFAS, LC-MS/MS

Laser Ablation combined with Electron Ionization-MS and ICP-OES for advanced polymer characterization

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Materials consisting of organic polymeric compounds are gaining importance due to their wide-ranging applicability and adaptability. Their versatility originates from their broad spectrum of chemical and physical properties that can be tuned and adjusted according to the intended purpose. These include a multitude of different applications in the industrial sector and daily life. When the required properties and functionalities cannot be achieved with one single material, polymeric composite materials are used. [1] For processing and recycling of polymeric materials, as well as for forensic analytics, reliable analysis methods are required to gain knowledge of the composition and thereby characterize the materials. This includes information such as the type of polymer, prevailing additives and potential contaminants.

Information about the organic composition of the sample can be gained by several conventional analysis techniques, such as LC-MS and GC-MS, that have the drawback of cumbersome sample preparation. Direct analysis methods for polymers such as Pyrolysis-GC-MS, FTIR and MALDI-MS require barely any sample preparation but show disadvantages ranging from tedious data processing and evaluation to ambiguities in the identification of samples consisting of more than one polymer. [2] The biggest drawback of these standard polymer analysis techniques is, however, that no simultaneous information on the elemental composition is available. For the complete characterization of polymer samples, knowledge of both the organic and inorganic constituents is necessary.

In this work, we present a novel methodology for advanced polymer analysis. The proposed approach is based on firing a focused laser beam onto a solid sample placed in an ablation cell. The generated gaseous and particulate ablation products are measured online and are detected simultaneously by EI-MS and ICP-OES. This multi-modal approach allows direct characterization of the main organic and inorganic components. EI-MS determines the molecular structure of the fragments that can be used as a fingerprint for a sample, even allowing polymer identification. ICP-OES is used to detect the contained inorganic sample constituents.

The concept of the proposed method was proven by classification experiments of polymer samples which could successfully be discriminated using the EI-MS data combined with ICP-OES data.

Keywords: Laser Ablation, Polymer analysis, Electron Ionization-MS, ICP-OES;

References:

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Session 2

12th May, 11:45 - 13:00, LC-MS & Metabolomics

6	11:45 – 12:00	**Utilization of Dual Column LC-HRMS to Assess of Below Ground Chemical Communication by Allelopathic Cover Crops** Alexandra Bennett, University of Natural Resources and Life Sciences, Institute of Analytical Chemistry, Vienna
7	12:00 – 12:15	**Utilization of high-resolution mass spectrometry in routine analysis of natural toxins** Lidija Kenjeric, FFoQSI – Austrian Competence Centre for Feed and Food Quality, Safety & Innovation,
8	12:15 – 12:30	**Development of a targeted LC-MS/MS method for the simultaneous detection of recently discovered bile acid conjugates** Daniel Wasinger, University of Vienna, Department of Food Chemistry and Toxicology
9	12:30 – 12:45	**Metabolomics of plasma and fecal samples obtained from a cohort of extremely premature infants** Manuel Pristner, University of Vienna, Department of Food Chemistry and Toxicology
10	12:45 – 13.00	**Investigating the Metabolome of *Trichoderma atroviride* and *Botrytis cinerea* under different light conditions using stable isotopic labelling** Kristina Missbach, University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology IFA-Tulln

Utilization of Dual Column LC-HRMS to Assess of Below Ground Chemical Communication by Allelopathic Cover Crops

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Growing cover crops is an effective way to manage soil and reduce weed propagation in agricultural settings. Resource competition is cited as a common way in which cover crops prevent weeds from developing early in the growing season[1]. However, the production of allelopathic compounds may give certain cover crop species a heightened advantage as these compounds are defined by their ability to impact the growth, reproduction, and development of other species. Non-targeted liquid chromatography (LC) high resolution mass spectrometry (HRMS) can be utilized to assess low molecular weight compounds released by the roots of previously identified [2] allelopathic cover crops in response to the presence of weedy neighbors.
Black oat (*Avena strigosa*) and buckwheat (*Fagopyrum esculentum*) cover crops were propagated in a split root setup on glass sand with Hoagland's nutrient solution with either (i) no neighbor, (ii) a homospecific neighbor, or (iii) heterospecific weedy neighbor redroot pigweed (*Amaranthus retroflexus*). Root exudates were extracted with a water fraction, plants were given 24 hours to regenerate metabolites, and again exudates were extracted with a methanol fraction. Extractions were concentrated and subsequently analyzed via a non-targeted dual column LC-HRMS method utilizing a quadrupole time of flight mass spectrometer (QTOFMS). Non-blank signals were annotated and differential expression between conditions was calculated.
Compounds from an in-house database (developed based on a mixture of 44 chemical standards) were identified in root exudate samples with a high degree (level 1) of confidence. The formula was annotated for the remaining compounds with MS FINDER[3] (Confidence level 4). Confidence levels were based on Schymanski *et al.* (2014).
After blank signal, noise filtration, and adduct aggregation, approximately 1000 to 1500 features remained in the aligned raw data sets for positive and negative mode for each condition. Differential analysis between growth setups was done by performing unpaired t tests on features that passed data filtration. Additionally, a fold change of 2 and 5 between different conditions was assessed.

Keywords: high resolution mass spectrometry (HRMS), metabolomics, allelopathy, cover crops

References:

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Utilization of high-resolution mass spectrometry in routine analysis of natural toxins

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Tandem mass spectrometry (MS / MS) is considered as a gold standard for targeted analysis of mycotoxins in complex matrices. However, there is a constant need for methods including plenty of analytes in a short time and overpass obstacles such as matrix effects in routine analysis. Here, high-resolution mass spectrometry emerges as a potential solution to all mentioned. Considering retrospective data mining, non-targeted data analysis, and shortening measurement times by fast polarity switching with high resolution, the utilization of HR-MS seems to be a step forward. The LC-MS / MS QTrap method for mycotoxin analysis with a dilute and shoot approach was transferred to an LC-Q Exactive HF HR-MS. Chromatographic conditions by Sulyok et al. 2020 remained identical within both approaches. Data were collected with QTrap 5500 MS / MS data in scheduled MRM mode in two individual chromatographic runs, in positive and negative ionization mode, respectively. Afterward, HR-MS data were acquired in the following full scan modes: (i) fast polarity switching, positive (ii) and negative ionization (iii) mode with resolving powers (i, ii, iii) of 120, 000 FWHM (at m / z 200) and (i, ii, iii) 240,000 FWHM (at m / z 200). For most analytes, the quality of obtained data did not suffer when fast polarity switching was used compared to individual ionization, as the number of points per peak ranged on average from 12 to 15. In addition, no significant difference was observed between the quantification limits by comparing those two modes. Furthermore, at high resolution detection limits improved. If comparing the linear range of the fast polarity switching mode on both resolutions, both resolutions > 90% of the analyte have a determination coefficient (R2) ≥ 0.99. Fast polarity switching mode at lower resolution showed to be the best setup to improve matrix effects since 87 % of analytes were in the signal suppression/enhancement (SSE) range of 80-120%. Against expectations, individual ionization had 80% of analytes in this range of SSE, while 70% of the analyte fell in the range if fast polarity switching mode at high resolution was applied. Thereby, the fast polarity switching mode at lower resolution improved the matrix effects the most. Moreover, fast polarity switching allows shortening the measurement time by half. The preliminary results showed that the application of LC-HRMS in multiple mycotoxin analysis with the option of retrospective data mining proved to be a competitive technique to LC-MS/MS.

Keywords: routine analysis, high-resolution mass spectrometry, hybrid triple quadrupole, linearity, sensitivity

References: Sulyok M. et al. 2020. doi: 10.1007/s00216-020-02489-9.

Development of a targeted LC-MS/MS method for the simultaneous detection of recently discovered bile acid conjugates

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The gut microbiome has long been associated with human health and disease through its metabolites affecting physiological functions of the host. One important example of these metabolites are secondary bile acids and bile acid conjugates, metabolized from primary bile acids by the gut microbiota. Recently, more than 100 new bile acid conjugates have been discovered of which some have a potential association with conditions such as IBD and obesity. Prompted by this, we batch-synthesized 120 conjugates, consisting of the newly discovered and already known conjugates, by linking proteinogenic amino acids with primary and secondary bile acids. After the successful tuning of the compounds' MS parameters, a targeted method using high-performance liquid chromatography and multiple-reaction monitoring on a triple quadrupole mass spectrometer was developed. An adequate separation of isobaric molecules was achieved, enabling the identification of all isomeric conjugates. The approach has been applied to biological samples including plasma, serum and feces. These proof-of-principle experiments demonstrate the potential of the developed assay for further exploring those newly discovered compounds and their potential association with disease.

Metabolomics of plasma and fecal samples obtained from a cohort of extremely premature infants

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The incidence of premature birth is one of the leading causes of perinatal mortality. While advancement in neonatal intensive care could increase the survival rate dramatically, a high number of surviving premature infants are afflicted by lifelong neurodevelopmental impairment. Being born on the edge of the third trimester, extremely premature infants and their neurological development are exposed to different environmental influences compared to an unborn fetus. One important element is the postnatal establishment of the microbiome in the gut and its derived metabolites.

The aim of this work is to investigate potential correlations between endogenous/exogenous metabolites, the formation of the gut microbiome, and neurophysiological development impairments in extremely premature infants. Mass spectrometry based untargeted metabolomics, employing hydrophilic interaction chromatography and reversed phase chromatography was used to measure over 200 fecal and plasma samples from extremely premature infants, exhibiting different degrees of neurodevelopmental impairment. Beforehand, a sample preparation procedure with a focus on usability with extremely small sample amounts and delivering reliable LC-MS measurements in long sequences was developed. After statistical data analysis, the unknown compounds of interest were annotated based on the results from MS^2 *in silico* fragmentation tools and molecular networking. Bile acids and respective conjugates, of which some were discovered just recently, were furthermore investigated in a targeted manner using LC-MS methods. This contribution will present the steps above in detail and highlight the insights obtained so far.

Keywords: Metabolomics, Infants, Gut microbiome, LC-MS

Investigating the Metabolome of *Trichoderma atroviride* and *Botrytis cinerea* under different light conditions using stable isotopic labelling

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Trichoderma atroviride (*Ta*) is a filamentous ascomycete which is widely used for biological control of plant diseases. A major mechanism used by *Ta* to antagonize plant pathogens like *Botrytis cinerea* (*Bc*) is mycoparasitism. Mycoparasitism involves the production of lytic enzymes and a variety of secondary metabolites.

This work is part of a project to study the interaction of *Ta* and *Bc* based on secondary metabolites produced by the two fungi in confrontation. Firstly, suitable cultivation conditions for the confrontation assay have to be established. Based on recent findings that the biosynthesis of 6-pentyl-α-pyrone (6-PP) and other volatile secondary metabolites by *Ta* are negatively affected by light, three light conditions have been tested: 1) complete darkness, 2) a 12/12 hours light/ dark cycle, and 3) occasional light exposure of up to a total of 15 min. These conditions were compared with respect to their effect on the secondary metabolome of the two fungi when they were cultured individually.
Fungal cultivation was performed on microscopic slides with agarose based minimal medium and glucose as sole carbon source (n=3 per condition) on either native (^{12}C) or $^{13}C_6$-glucose. After 72 hours, starting from the inoculation spot, the agarose was cut into 1 cm-wide stripes and the therein contained metabolites analyzed. This also allowed to study metabolite mobility and their rough localization.
Samples were quenched in liquid nitrogen and extracted with acetonitrile/water (1:1, v/v). All U-^{13}C labeled extracts were pooled and mixed with ^{12}C-extracts of individual culture stripe samples, followed by measurement by RP-LC-HRMS. Labeling-specific isotope patterns were extracted from the raw data, to assign all *Ta*-derived metabolites using the MetExtractII software.
First data evaluation showed that the majority of the metabolites were found across all biological replicates, for complete darkness, 12/12 h light/darkness and the randomly light exposed samples, respectively. While many substances, including 6-PP, were significantly more abundant after cultivation in complete darkness, a number of metabolites turned out to be formed under specific conditions only. The presentation gives an overview of the global metabolomes as well as the location of the detected compounds on the culture slides.
In conclusion, the study revealed that the vast majority of the detected compounds can be found ahead of the hyphal growth front and that occasional light exposure (max. 15 min in total) instead of complete darkness, is a suitable condition for our future mycoparasitic confrontation experiments.

Keywords: Untargeted Metabolomics; High-resolution-mass-spectrometry; Fungal Metabolomics; Secondary Metabolites

Session 3

12th May, 14:00 – 15:00, NIR & AFM-IR

11	14:00 – 14:15	**Portable vs. Benchtop NIR-Sensor Technology for Classification and Quality Evaluation of Black Truffle** Christoph Kappacher, Institute of Analytical Chemistry and Radiochemistry; Leopold-Franzens University Innsbruck
12	14:15 – 14:30	**Inline Process Monitoring in the Production of Polyhydroxy-alkanoates with fully-integrated Near-Infrared Spectroscopy** Sebastian Friedl, RECENDT-Research Center for Non Destructive Testing, Linz, Austria
13	14:30 – 14:45	**Nanoscale characterizing of organic-inorganic perovskites with AFM-IR** Ufuk Yilmaz, Institute of Chemical Technologies and Analytics, TU Wien
14	14:45 – 15:00	**Modelling thermal expansion as a point spread function for nanoscale chemical imaging** Yide Zhang, Institute of Chemical Technologies and Analytics, TU Wien

Portable vs. Benchtop NIR-Sensor Technology for Classification and Quality Evaluation of Black Truffle

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Truffles represent the best known and most expensive edible mushroom. Known as *Ascomycetes*, they belong to the genus *Tuber* and live in symbiosis with plant host roots. Due to their extraordinary taste and smell, truffles are sold worldwide for high prices up to 3000 – 5000 euros per kilogram (Tuber magnatum PICO). Amongst black truffles, the species *Tuber melanosporum* VITTAD. is highly regarded for its organoleptic properties. Nonetheless, numerous different sorts of black truffle are offered at lower prices including *Tuber aestivum* VITTAD., *Tuber indicum* and *Tuber uncinatum*, which represent the most frequently consumed types. Since truffles visually do not differ for inexperienced consumers, food fraud is likely to occur. Especially for high-prized *Tuber melanosporum*, forming morphologically very similar fruiting bodies to *Tuber indicum*, a risk of fraud through imported truffle from Asia is given. 126 truffle samples belonging to the four mentioned species were investigated by four different NIR-instruments, including three miniaturized devices such as Tellspec Enterprise Sensor, VIAVI solutions MicroNIR 1700 and Consumer Physics SCiO, working on different technical principles. Three different types of measurement techniques were applied for all instruments (outer shell, rotational device and fruiting body) in order to identify the best results for classification and quality assurance in a non-destructive way. Results provide differentiation with an accuracy up to 100% for the expensive *Tuber melanosporum* from *Tuber indicum*. Classification between *Tuber melanosporum*, *Tuber indicum*, *Tuber aestivum* and *Tuber uncinatum* could also be achieved by 100%. In addition, quality monitoring including discrimination between fresh and frozen/thawed, as well as prediction of the approximate date of harvesting was performed. Furthermore, feasibility studies according to the geographical origin of the truffle have been attempted. The presented work compares the performance for prediction and quality monitoring of portable vs. benchtop NIR-devices and applied measurement techniques in order to be able to present a suitable, accurate, fast, non-destructive and reliable method for consumers.

Keywords: black truffle; near-infrared spectroscopy; chemometrics; food quality; food adulteration

Inline Process Monitoring in the Production of Polyhydroxy-alkanoates with fully-integrated Near-Infrared Spectroscopy

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Polyhydroxyalkanoates (PHAs) are a large group of polyesters with increasing relevance in the field of bio-polymers. They can be produced from renewable sources containing cellulose and are biodegradable in normal atmosphere and under water. PHAs have the potential to replace common petrochemical polymers as packaging and coating material due to their similar material properties. However, their relatively high production costs are limiting their applicability [1]. In this work we present the use of cost-efficient near infrared (NIR) spectrometer technology based on micro-opto-electromechanical systems (MOEMS) to develop multivariate chemometric models which can be used to improve the production process of the polymer.
PHAs are produced in three steps. In the first step levulinic acid is produced from cellulose in a high-pressure reactor. The current reactor design did not allow the direct acquisition of inline spectra. Thus, samples were taken every hour and analysed using a typical NIR immersion probe, thereby simulating inline measurement conditions. Reference data have been provided by high-performance liquid chromatography (HPLC). With the HPLC data a partial least squares (PLS) regression model has been calculated, which can predict the concentration of glucose and levulinic acid within the process samples. This model was later validated by an independent experiment. The root mean square error of prediction (RMSEP) for levulinic acid was 1.62 g/L and 1.83 g/L for glucose.
In the second step, the bacterium *Cupriavidus necator* is fed with levulinic acid and acetic acid to produce a poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) polymer. The ratio of 3-hydroxy-butyrate and 3-hydroxyvalerate is determined by the ratio of levulinic acid and acetic acid. During the process, NIR spectra were taken directly through the glass wall of the employed bioreactor using the built-in light sources of the NIR spectrometer modules, thus eliminating the need for an expensive immersion probe, and significantly reducing the risk of contamination [2]. The collected spectra were used to create a PLS model to predict the optical density and the concentration of copolymer in the reactor.
In the last production step, the polymer is extruded. During the process a special extruder probe and an NIR spectrometer were used to gain information about the changes in the absorption spectra between different processes. The obtained data showed a promising correlation to key material properties like viscosity and crystallinity of the final product.

Keywords: Polyhydroxyalkanoates, near-infrared, spectroscopy, process monitoring, chemometrics, partial least squares regression, micro-opto-electromechanical systems

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Nanoscale characterizing of organic-inorganic perovskites with AFM-IR

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Organic-inorganic, hybrid perovskite technology is regarded as a contender for the next generation of lighting and light harvesting technologies. Perovskite LEDs (PeLEDs) and perovskite photovoltaics (PePV) are cheap, light, flexible, efficient and easy to process and manufacture. However, currently, perovskite devices suffer from rapid degradation through various failure modes (humidity, UV aging, heat,..). Pe technology offers several avenues to overcome these issues by adjusting the chemical composition (metal cation, halogen anion, organic cation, matrix components and stabilizers) of devices. Research into PeLED/PePV synthesis und fabrication has been going on for two decades. To understand these failure modes and the effects of changes to the composition on the device performance macroscale chemical characterization is insufficient. Instead, characterization at the nanoscale is required to understand and optimize PeLEDs/PePVs. In our current research we study novel perovskite materials by combining scanning probe microscopy to achieve nanoscale resolution with mid-IR spectroscopy for chemical identification (AFM-IR). The working principle is based on leveraging a local, short-lived photo-thermal expansion by absorption of infrared light - induced by a pulsed, tunable EC-QCL source. This excitation then is measured by the cantilever probe and the oscillation amplitude is directly proportional to the absorption and thus an absorption spectrum is generated. Using this nearfield IR spectroscopy technique, we study novel perovskite materials to understand their phase composition at nanometer spatial resolution. In using AFM-IR to gather mid-IR absorption spectra from sample areas smaller than a few tens of nanometers we can see chemical changes within a single perovskite crystallite. Using hyperspectral nanoscale chemical imaging we can collect images of the distribution of perovskites and stabilizers and thus provide information required to design the next generation of lighting and light harvesting devices.

Keywords: infrared spectroscopy; nanoscale; nearfield; characterization.

Modelling thermal expansion as a point spread function for nanoscale chemical imaging

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Atomic force microscopy-infrared (AFM-IR) is an AFM based technique that measures mid-IR absorption spectra at nanometre spatial resolution. The technique of AFM-IR relies on the detection of the pulsed wavelength tuneable IR laser induced thermal expansion of the sample area underneath the AFM tip. While this mode of signal generation sounds simple enough it is still not fully understood.

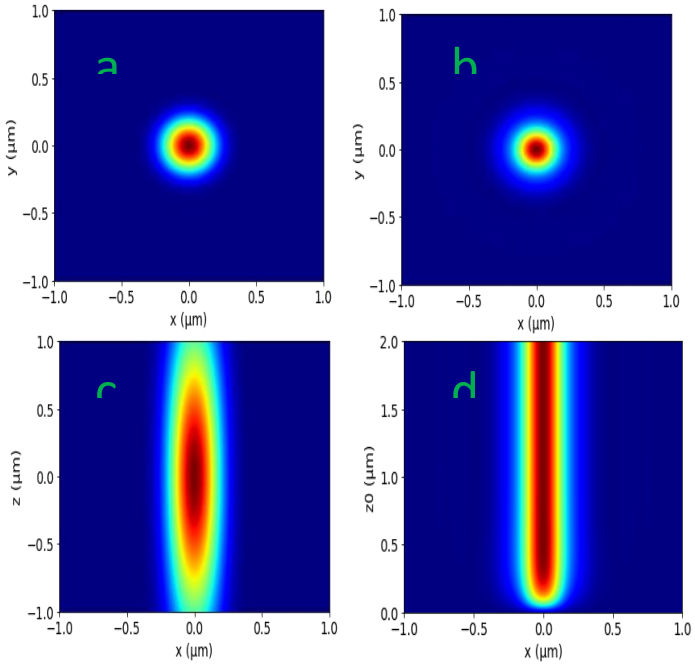

Figure 1. Comparison of point spread function (PSF) in confocal imaging at numerical aperture 1.0 and wavelength 488nm and AFM-IR. PSF of confocal imaging **a** in x-y plane and **c** in x-z plane. PSF of AFM-IR **b** in x-y plane and **d** in x-z plane.

In this work, we present a theoretical investigation of the laser heating induced thermal expansion process and model it as a point spread function (PSF). This approach draws parallels to super resolution microscopy where the PSF is used to determine spatial resolution and to resolve features below the diffraction limit (as shown in figure 1). By solving the inhomogeneous heat equation with a volumetric heat source, we obtain the PSF of AFM-IR in frequency domain displays as,

$$|T(r,z_0,f)| = \frac{4a\alpha g_v}{\pi\kappa L^2 b^2}\sum_{n=0}^{\infty}\sum_{m=0}^{\infty}\frac{J_0(\beta_m r)}{J_1^2(\beta_m b)\beta_m\eta_n^2}J_1(\beta_m a)sin(\eta_n a)sin(\eta_n z_0)\sqrt{\frac{2}{(2\pi f)^2+\alpha^2\lambda_{nm}^4}}\frac{|sin(\pi f t_p)|}{f}$$

Eqn. 1

Where α is the thermal diffusivity, κ is the heat conductivity, g_v the volumetric heat source density which linked to the laser heating. To verify that simplified boundary conditions do not result in a significantly changed behaviour a finite element model **of** heat conduction in solids and fluids (implemented in COMSOL Multiphysics 5.6) of more realistic sample geometries is used. In this case we consider a cylindrically shaped sample with a single spherical element thermally heated by a laser pulse. The sample is placed on a thick substrate and covered by an air layer. Finally, the theoretical considerations yielding the PSF are compared to COMOSL simulated data.

First results how that there is a frequency (pulse repetition rate), pulse length and sample geometry dependence of the PSF in AFM-IR. The achievable spatial resolution is improved for short pulses, high frequencies, small absorber size and when the absorbers are closer to the substrate. These results provide guidance for experimental parameters which can be considered as trade-off between spatial resolution and signal intensity.

Session 4

12th May, 15:30 – 16:45, Environment& Biotechnology

15	15:30 – 15:45	**Polycyclic aromatic hydrocarbons and where to find them: highlights and obstacles of the development of analytical methods for PAH quantification in different environmentally important matrices** Bernadette Kirchsteiger, TU Wien, Institute of Chemical Technologies and Analytics
16	15:45 – 16:00	**Analysis and purification of ice nucleating macromolecules from birch pollen leading to new insights of their properties** Florian Reyzek, Institute of Materials Chemistry, TU Wien
17	16:00- 16:15	**MALDI-intact cell mass spectrometry as a monitoring tool for fermentation processes** Cristian Zanetti, TU Wien, Institute of Chemical Technologies and Analytics
18	16:15 – 16:30	**^{13}C-tracer based metabolomics reveals a hidden methanol and CO2 assimilation pathway in the methylotrophic yeast *Pichia pastoris*** Bernd Mitic, Institute of Microbiology and Microbial Biotechnology, University of Natural Resources and Life Sciences, Vienna
	16:45 - 17:45	**Poster Session**

Polycyclic aromatic hydrocarbons and where to find them: highlights and obstacles of the development of analytical methods for PAH quantification in different environmentally important matrices

Bernadette Kirchsteiger*[a], Hanne Ø. Notø[b], Dušan Materić[b], Sebastian Wöhrer[a], Karoline Rieger[a], Rupert Holzinger[b] and Anne Kasper-Giebl[a]

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Anthropogenic air pollution states one of the greatest environmental risks in modern times, with multiple facets related to economic, political and social concerns [1-3]. Although the European Union strongly promotes the use of biomass to replace fossil fuels in order to reach the Paris Agreement, emissions from residential wood combustion have been identified as a major contributor to the local air pollution in Europe [4] and thus also in Austria [5, 6]. Besides particulate emissions, wood combustion is responsible for the emission of various toxic substances, like polycyclic aromatic hydrocarbons (PAHs). PAHs are persistent and widespread substances, comprising a group of several hundred congeners which are always emitted as a complex mixture. In recent times, many of these ubiquitous PAHs gained special interest because of their known toxic and carcinogenic effects [7].

This talk highlights the development of different analytical procedures to quantify PAHs from environmentally important matrices and the obstacles to overcome. Concentrations of PAHs show a gradient trend in different compartments and highest contributions were found in emission and ambient samples [6, 8], for which already standardized analytical methods are available. To understand in which extend PAHs are dispersed and transported from one region to another, we enlarged the common focus of PAH contributions to other vehicles for atmospheric transport, i.e. snow and cloud water. Shifting the focus to different environmental compartments is entailed with a couple of analytical challenges requiring a multi-method approach. In general, lower PAH contributions are expected in snow and cloud water samples, they, however, significantly differ in the available sample volume. For PAH quantification in snow samples, we are working on a method for the parallel quantification of up to 33 PAHs based on a solid-phase extraction sample preparation procedure followed by a more sensitive GC-MS/MS analysis. When it comes to cloud water samples, the combination of low sample volume and analyte concentration refuses the use of typically used analytical methods for PAH quantification. To circumvent this challenge, we developed a novel method based on thermal desorption-proton transfer reaction – mass spectrometry (TD-PTR-MS). This project was funded by the Lions Sponsorship and realized in collaboration with colleagues from Utrecht University.

Keywords: Polycyclic aromatic hydrocarbons, ambient PM, snow, cloud water

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Analysis and purification of ice nucleating macromolecules from birch pollen leading to new insights of their properties

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Understanding the Earth's climate and its influencing factors is becoming increasingly important, yet the influence of aerosol particles on the climate is still uncertain. Numerous aerosols, such as dust, soot, and biological particles, can act as ice nuclei (IN) and trigger the freezing of supercooled liquid cloud droplets, thus changing cloud properties, the albedo, and the formation of precipitation.
Ice nuclei of biological origin, like bacteria, fungi, or pollen, can have remarkably high onset freezing temperatures. Pummer et al. (2012) found that solubilized macromolecules are responsible for the ice nucleation activity of tree pollen and not the grains themselves. More recently, ice-nucleating macromolecules (INMs) have also been found on other tree tissues (Felgitsch et al., 2018). In general, INMs are present in much larger numbers than the micrometer-sized pollen grains and thus the emission of INMs from the biosphere might play a more important role than previously thought.
Still, the chemical composition and structure of INMs remain largely unknown. To shine light on this, we extracted INMs from birch pollen with water. In order to concentrate the extracted INMs we used ice affinity purification, which enabled us to perform further characterization experiments with INM at varying concentrations.
We found ice nucleation activity of birch pollen INMs at temperatures of up to -5°C, which is much higher than the -15°C previously reported by many studies. We further detected ice nucleation activity after filtration through a 10 kDa cutoff filter, which is much smaller than the previously reported 100 kDa. Lastly, we found the INMs to be sensitive to heat as the ice nucleation activity decreases with increased temperature and duration of heat treatments. Together with further analytics, like CD and fluorescence spectroscopy, this led us to the hypothesis that the INMs are made up of aggregates and might be proteinaceous.
All our results especially the discovered ice nucleation activity at -5°C could necessitates a substantial revision of our view of tree pollen INMs as contributors to atmospheric ice nucleation.

Keywords: ice nucleation, birch pollen, Ice affinity purification, INMs, bioaerosols.

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MALDI-intact cell mass spectrometry as a monitoring tool for fermentation processes

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MALDI-Intact Cell Mass Spectrometry (MALDI-ICMS) is a helpful technique for discriminating/identifying microorganisms (MALDI Biotyping); it is used in clinics for rapid pathogen identification in case of hospital-acquired infections (e.g., Staphylococcus aureus), enabling quick response and intervention. Industrial fermentations exploit microorganisms for synthetizing products of interest; while the failure of such fermentations is cost-intensive, process characterization can help decision-making for process optimization. Aiming at developing novel process monitoring tools, we present the use of MALDI-ICMS to monitor E. coli fermentations.

Samples from E. coli bioreactors were collected at different fermentation times, aliquots were washed by pelletation and resuspension in a NH4HCO3 buffer (pH 7) and equalized to the same OD550, the cell suspensions were mixed with a MALDI matrix at a ratio of 1:2 (sinapinic acid/ferulic acid, 0.5 % trifluoroacetic acid, 70 % acetonitrile). The mixture was spotted in replicates on a MALDI target and air dried. The samples were analyzed on a MALDI-TOF device (UltrafleXtreme, Bruker) in the positive linear mode (2-20 kDa mass range). By using multivariate data analysis in the R environment, we exploited time-dependent spectral changes to build a model capable of monitoring the fermentation progress. While principal component analysis could separate fermentation phases, partial least square regression could track fermentation progress with a prediction error of less than 5 % of the total fermentation time. The presented approach will allow better early-stage process characterization and prediction of process outcome.

Keywords: MALDI MS, Intact Cell Mass Spectrometry, Biostatistics

^{13}C-tracer based metabolomics reveals a hidden methanol and CO_2 assimilation pathway in the methylotrophic yeast *Pichia pastoris*

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The yeast *Pichia pastoris* (*Komagataella phaffii*) can grow on methanol as the sole carbon and energy source for biomass and protein production. Using ^{13}C-methanol labeling and reverse labeling combined with advanced GC-HRMS and LC-IM-QTOFMS metabolomics methodologies, we revealed an alternative methanol assimilation pathway, which co-assimilates CO_2.

The main xylulose 5-phosphate pathway for methanol assimilation in yeast was deleted by knocking out *DAS1* and *DAS2* with CRISPR/Cas9. Besides this pathway, there are alternative methanol assimilation pathways in prokaryotes, which can co-assimilate CO_2. Possible pathways are the serine cycle and the reductive glycine pathway. In both pathways methanol is dissimilated to formate, which is then fixated by the tetrahydrofolate cycle to form methylenetetrahydrofolate. Glycine is provided by *de-novo* synthesis via reaction of methylenetetrahydrofolate with CO_2 or produced by the serine cycle. A methyl group transfer from methylenetetrahydrofolate to glycine leads to serine, which is then used as a precursor for all biomass formation.

Isotopologue distribution analysis of intracellular metabolites in combination with ^{13}C-methanol labeling or reverse labeling was applied to examine pathway activities. As metabolism is strongly altered in the knockout strains, GC-TOFMS methods applicable for the wild type had to be further developed to cope with the interfering background. To cover all important amino acids, organic acids, sugars, and their phosphorylated analogues, three different GC-TOFMS methods were applied, using different ion sources, derivatization agents and on-column concentrations. To evaluate the labeling degree in the folate pathway, a fit-for-purpose folate extraction method was developed, as folates are instable and prone to interconversion. Selective analysis was subsequently performed with LC-IM-QTOFMS.

Based on the isotopologue distribution analysis results, we reveal that the reductive glycine pathway is active which was further validated using targeted knockouts and overexpressions of this pathway.

Session 5

Session 1, 13th May, 09:45 – 10:45, Miscellaneous

19	09:45 – 10:00	**Fluorescence labeling of C1 oxidized cellulose: Method development** David Budischowsky, Institute of Chemistry of Renewable Resources, University of Natural Resources and Life Sciences, Vienna
20	10:00 – 10:15	**Extracellular vesicle characterization via nano-electrospray gas-phase electrophoretic mobility analysis (nES GEMMA)** Stephanie Steinberger, TU Wien, Institute of Chemical Technologies and Analytics
21	10:15 – 10:30	**A new method for the determination of cobalamins in mushrooms** Martin Walenta, Institute of Chemistry – Analytical Chemistry, University of Graz
22	10:30 – 10:45	**Liquid-phase microextraction of cannabidiol from cosmetics by means of recyclable ionic liquids** Susanne Huber, Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University of Innsbruck

Fluorescence labeling of C1 oxidized cellulose: Method development

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A novel method for fluorescence labeling and subsequent profiling of oxidized end groups along the molar mass distribution of cellulose has been developed. A coupling method using carbodiimides to bind the fluorophore *N*-(1-naphthyl)ethylenediamine (EDAN) to the respective oxidized site of the model compound cellobionic acid has been established and optimized regarding the reaction conditions and the presence of a catalyst. The reaction product, EDAN-labeled cellobionic acid yielded 98% and was isolated by flash chromatography and characterized by NMR spectroscopy and MS confirming the structure and purity of the product. CBE was used as a standard material for HPLC quantification for the reaction kinetics and stability analysis. The optimized labeling conditions were adjusted for the heterogeneous derivatization of cellulose followed by GPC MALLS/IR analysis combined with fluorescence detection.

Keywords: Aldonic acid; Cellulose; Fluorescence labeling; HPLC, GPC

Extracellular vesicle characterization via nano-electrospray gas-phase electrophoretic mobility analysis (nES GEMMA)

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Extracellular vesicles (EVs) are emerging as future therapeutics and clinical diagnostic tools indicating a human's health status. They are formed and released by cells into their surroundings for e.g., cell-to-cell communication, blood coagulation or even tumor metastasis. However, their heterogeneity and diversity in size and function makes their isolation and characterization difficult.

For future medical applications purity of EV-containing samples is of importance. In this context, samples were analyzed via nano electrospray gas-phase electrophoretic mobility analysis (nES GEMMA) separating single-charged (bio)nanoparticles in the gas-phase at ambient pressure according to electrophoretic mobility (EM) diameters. nES GEMMA proved to be a reliable characterization technique for liposomes, strongly resembling EVs in constitution. In comparison to prevalent techniques like nanoparticle tracking analysis (NTA), determining the hydrodynamic particle diameter, nES GEMMA allows the determination of the surface dry particle diameter with particle-number based detection. Hence, larger molecules can be monitored next to smaller ones, independent of the chemical composition of an analyte. For nES GEMMA the EV sample buffer PBS was exchanged to a volatile ammonium acetate, necessary for nES-based measurements. nES GEMMA of EV-containing samples displayed a broad particle distribution, starting around 20 nm and tailing off to larger EM diameter values. Impurities, mostly co-isolated proteins, were detected in the lower EM diameter range (<20 nm). NTA corroborated the presence of vesicles and their loss during sample preparation in samples but failed to report those impurities despite high particle counts in nES GEMMA. For complete analysis these components were identified by mass spectrometry as hemoglobin, α-2-macroglobulin, and β-actin, correlating with the size/molecular mass calculations based on nES GEMMA (Steinberger et al., 2021, *Anal. Bioanal. Chem.*).

An additional size exclusion chromatography (SEC) step after EV isolation depleted the mentioned impurities, evident in nES GEMMA spectra. The overall EV hydrodynamic size distribution and sample proteome was not influenced by SEC, verified by NTA and MS analysis. However, SEC contributes to a vesicle number loss and leads to an increased number of spikes in the spectra due to lipid fragments, indicating rupture and damage to vesicles.

In conclusion, nES GEMMA is suitable for EV characterization and purity assessment, revealing co-isolates proteins not detectable via NTA. Additional SEC improves sample purity at a loss of vesicle numbers.

This work was supported by the NÖ Forschungs- und Bildungsges.m.b.H (NFB), grant LSC16-018.

Keywords: Extracellular vesicles, EV characterization, nES GEMMA, nDMA

A new method for the determination of cobalamins in mushrooms

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Vitamin B_{12} is essential for the human body and has to be part of our nutrition. It is mainly found in meat, seafood and dairy products and therefore a vegetarian lifestyle risks a deficiency. Mushrooms are often mentioned as a possible source of vitamin B_{12}. Determination of vitamin B_{12} in mushrooms is carried out spectrophotometrically or with electrospray ionization mass spectrometry focusing on cyano-cobalamin.[1,2] To verify the concentrations of all biologically active metabolites in commonly eaten mushrooms we developed a method for the determination of the different metabolites of vitamin B_{12} using high performance liquid chromatography (HPLC) coupled to inductively coupled mass spectrometry (ICPMS). Published methods are often time consuming and 15 minutes were typically needed to separate the vitamin B_{12} metabolites.[3] To reduce the chromatographic runtime we employed dimethyl carbonate (DMC) as an eluent providing faster elution and favoring the sensitivity of the ICPMS detection.[4] For the separation a reverse-phase column with a mobile phase containing ammonium acetate buffer and DMC were used. A suitable separation of all tested cobalt compounds, namely inorganic Co(II), cyanocobalamin (CN-Cbl), hydroxocobalamin (OH-Cbl), 5´-deoxyladenosylcobalamin (Ado-Cbl) and methylcobalamin (Me-Cbl) could be achieved in less than 8 minutes. The optimized method was applied to mushroom samples.

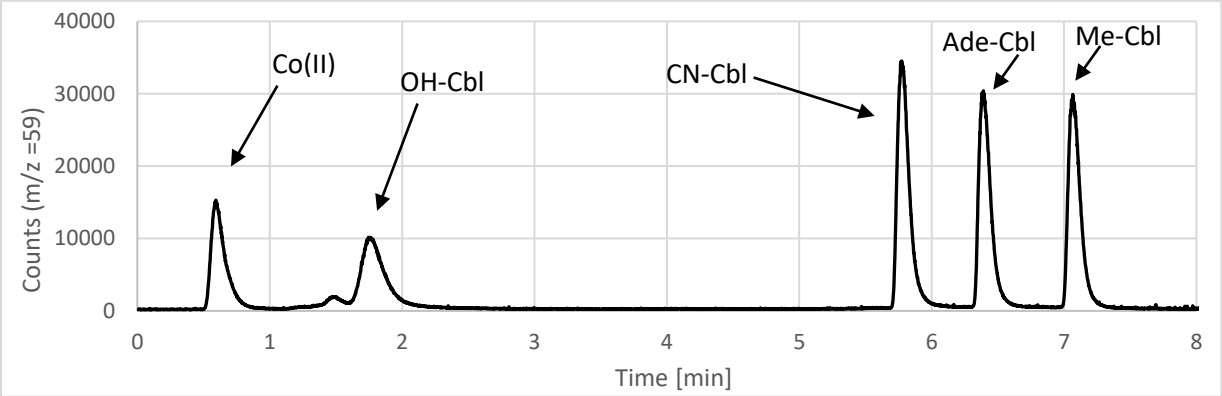

Figure 1 HPLC-ICPMS chromatogram of inorganic Co(II) and Vitamin B_{12} metabolites

Keywords: vitamin B_{12}, Co-speciation, mushrooms, HPLC-ICPMS

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Liquid-phase microextraction of cannabidiol from cosmetics by means of recyclable ionic liquids

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An innovative liquid-phase microextraction technique was developed for the isolation of cannabidiol from different natural cosmetics, especially creams and salves. Therefore, a novel synthesized imidazolium triflimide based room temperature ionic liquid (RTIL), was used as an extracting agent. Quantification was implemented, using a high performance liquid chromatography system coupled to ultraviolet detection. The extraction procedure was optimized by means of two different design of experiments. Additionally, a full validation was executed. Accuracy and precision on four consecutive days as well as a linear calibration model, ranging from 0.6 to 6.0 mg g^{-1}, were confirmed. Recoveries, tested for low and high concentration within the calibration range, were about 80%. Stability of extracted cannabidiol was proven for three days at room temperature and fourteen days at 4 °C and -20 °C. Liquid-phase microextraction of cannabidiol from different formulated cream based cosmetics was performed, including ointments and creams. The results show that a significantly higher selectivity could be achieved compared to conventional extraction methods such as with methanol. In the next step, the method was extended to enable recovery of cannabidiol from the extracting agent followed by RTIL recycling. Hence a solid-phase extraction procedure was developed, including three different imidazolium triflimide RTILs as sample matrices. Nine commercially available solid sorbents were tested for their analyte recovery and RTIL separation performance, with three sorbents being selected and applied for further detailed extraction experiments. Final results showed high recoveries of up to 97.8% for the extraction of cannabidiol. After analyte extraction, 93.3 to 96.5% of RTIL could be recycled without residual contaminations. Conclusively, the enhancement of the method creates the opportunity to sustainably utilize RTILs in analytical sample preparation.

Keywords: cannabidiol, room temperature ionic liquid, ionic liquid reuse, cosmetic products, sample preparation

Session 6

13th May, 11:15 – 12:15, Mid-IR

23	11:15 – 11:30	**Mesoporous Zirconia Coating for Sensing Applications Using Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) Spectroscopy** Dominik Wacht, Institute of Chemical Technologies and Analytics, Technische Universität Wien
24	11:30 – 11:45	**Application of Laser-Based Mid-Infrared Spectroscopy for Inline Monitoring of Proteins from Preparative Liquid Chromatography** Christopher Karim Akhgar, Institute of Chemical Technologies and Analytics, Technische Universität Wien
25	11:45 – 12:00	**Fast investigation of chirality: Balanced detection and Quantum Cascade Lasers for improved Vibrational Circular Dichroism** Daniel-Ralph Hermann, Institute of Chemical Technologies and Analytics, TU Wien, Vienna
26	12:00 – 12:15	**Mid-IR dispersion spectroscopy for chemical analysis in the liquid-phase** Alicja Dabrowska, Institute of Chemical Technologies and Analytics, Technische Universität Wien

Mesoporous Zirconia Coating for Sensing Applications Using Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) Spectroscopy

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Mid-infrared attenuated total reflection (ATR) spectroscopy is a powerful tool for in situ monitoring of various processes. Mesoporous silica, an extensively studied material, has already been applied in sensing schemes due to its high surface area and tunable surface chemistry. However, its poor chemical stability in aqueous solutions at pH values higher than 8 and strong absorption below 1250 cm^{-1} limits its range of applications. To circumvent these problems, a mesoporous zirconia coating on ATR crystals was developed. Herein, the synthesis, surface modification, and characterization of ordered mesoporous zirconia films on Si wafers and Si-ATR crystals are presented. The modified coating was applied in sensing schemes using aromatic and aliphatic nitriles in aqueous solution as organic pollutants. The mesoporous zirconia coating shows strong chemical resistance when kept in alkaline solution for 72 h. The success of surface modification is confirmed using Fourier transform infrared (FT-IR) spectroscopy and contact angle measurements. Benzonitrile and valeronitrile in water are used as model analytes to evaluate the enrichment performance of the film. The experimental results are fitted using Freundlich isotherms, and enrichment factors of 162 and 26 are calculated for 10 mg L^{-1} benzonitrile and 25 mg L^{-1} valeronitrile in water, respectively. Limits of detection of 1 mg L^{-1} for benzonitrile and 11 mg L^{-1} for valeronitrile are obtained. The high chemical stability of this coating allows application in diverse fields such as catalysis with the possibility of in situ monitoring via FT-IR spectroscopy.

Keywords: porous materials, infrared spectroscopy, functional coatings, sensor, thin film

Application of Laser-Based Mid-Infrared Spectroscopy for Inline Monitoring of Proteins from Preparative Liquid Chromatography

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Laser-based mid-infrared (IR) spectroscopy is an emerging technique for analyzing proteins in aqueous solutions. Novel spectrometers based on external cavity-quantum cascade lasers (EC-QCLs) offer significant advantages compared to gold-standard Fourier-transform IR (FTIR) instrumentation such as higher sensitivity, larger applicable optical path-lengths and increased ruggedness. These advantages open a wide range of possible applications, including measurements of proteins from complex purification operations.

In this work, a commercial EC-QCL based mid-IR spectrometer was applied for inline monitoring of proteins from preparative liquid chromatography (LC). The large optical path length (25 µm) of the equipped transmission cell and the broad tuning range of the laser (1350-1750 cm^{-1}) enabled robust spectra acquisition in the most important wavenumber region for protein secondary structure analysis. To demonstrate the advantages of QCL-IR spectroscopy over conventional LC detectors, two different purification operations based on ion-exchange chromatography (IEX) and size exclusion chromatography (SEC) were monitored.

In IEX, a major challenge was caused by the applied sodium chloride gradient, inducing mid-IR absorbance bands that overlap with protein bands and dominate to recorded spectra. Here, a novel background compensation approach was implemented to eliminate salt bands, leading to high quality protein spectra. In case of SEC, proteins with similar molecular weights, leading to overlapping chromatographic peaks that cannot be distinguished with conventional UV detectors were monitored. Here, the combination of laser-based mid-IR spectroscopy and chemometrics allowed estimation of individual protein concentrations across the chromatographic run based on their secondary structure. For both systems, the obtained results were compared to reference high-performance LC (HPLC) offline measurements, showing excellent agreement in terms of protein identification as well as quantification.

Consequently, QCL-IR spectroscopy can be successfully applied for inline detection of proteins from LC effluents, providing information that is typically only accessible by time and cost-intensive offline methods.

Keywords: Mid-infrared spectroscopy, quantum cascade laser, liquid chromatography, proteins, secondary structure

Fast investigation of chirality: Balanced detection and Quantum Cascade Lasers for improved Vibrational Circular Dichroism

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Chirality is an integral part of chemistry, being present in small molecules and biological macromolecules like proteins.[1] It is readily accessible by chiroptical spectroscopy techniques, like Circular Dichroism (CD) and Vibrational Circular Dichroism (VCD). Unlike CD, which relies on the presence of specific chromophores in the analyte, VCD operates in the infrared region, which is rich in bands characteristic for most organic molecules. Unfortunately, besides the broad applicability, VCD is also characterised by weak signal amplitudes (10^{-5} – 10^{-6} compared to classical absorbance), which can easily be distorted by birefringence artifacts originating in the optical components of the instrument.[2] High quality VCD spectra therefore are collected over long measurement times (up to several hours) to achieve reasonable signal-to-noise ratios.
Quantum Cascade lasers (QCLs) provide highly polarised light in the infrared region with a high spectral power density. These characteristics lend themselves well to VCD, possibly facilitating fast measurements while still obtaining low noise levels. Impressive results were already achieved for classical IR-spectroscopy, with QCL-based setups outperforming FT-IR spectrometer in terms of noise levels.[3] We aimed to achieve similar results also for VCD by augmenting a QCL based VCD setup by a balanced detection scheme. This allowed us to compensate for the noise present in QCLs, such as pulse to pulse fluctuations and thermal drifts.[3] We were able to acquire VCD spectra with measurement times below 10 minutes, while still outperforming FT-IR instruments in terms of noise levels by factors up to 6. Additionally, the properties of the used External Cavity -QCL (EC-QCL) would allow us to decrease the measurement time further, if a smaller spectral range is needed. We applied this improvement on an enantiomeric excess study of R/S-1,1′-Bi-2-naphthol in $CHCl_3$ with measurement times below 5 minutes. We are confident that this scheme will enable improved applicability of VCD as a process analytical tool in chiral reactions and bioprocesses.

Keywords: Vibrational Circular Dichroism, Quantum Cascade lasers, infrared spectroscopy

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Mid-IR dispersion spectroscopy for chemical analysis in the liquid-phase

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Mid-infrared (mid-IR) spectroscopy is a widely used and powerful analytical technique allowing for highly selective, sensitive, and non-destructive sample investigation by probing molecular vibrations. The introduction of quantum cascade lasers (QCLs) as coherent and inherently polarized sources of mid-IR radiation enabled new spectroscopy schemes that go beyond classical absorption spectroscopy.

Measurements of the change in refractive index (dispersion; phase shifts) rather than absorption (light attenuation) induced by an absorbing analyte is one alternative approach to classical spectroscopic techniques. Dispersion sensing delivers quantitative and qualitative information about the sample equivalent to absorption spectroscopy with the advantages of immunity to source intensity fluctuations, high dynamic range, and baseline-free detection. Whereas QCL-based dispersion spectroscopy of gaseous-samples is an established method, it is still an emerging approach for analysis of liquid-phase samples.

In this work, a compact setup for mid-IR dispersion spectroscopy of liquids based on an external cavity-QCL coupled to a Mach-Zehnder Interferometer is presented and its operating principle is explained. This newly shown configuration features a fast hysteresis-free piezo-actuated mirror for reliable phase-locked interferometric detection at the quadrature point that proves to have tremendous impact on the sample's dispersion sensing, fully unlocking its previously-mentioned advantages, and improving the reproducibility, robustness, and limits of detection of the method. A new routine for fast and almost simultaneous acquisition of real and imaginary part of the refractive index (i.e., dispersion and absorption spectra) was implemented for mutual spectra validation. The setup's high thermal and mechanical stability was achieved by extensive temperature and environmental control, setup miniaturization, and the implementation of a new dual-channel transmission cell.

Our showcases demonstrate the power of the technique and the developed setup for quantitative and qualitative analysis of various analytes (i.e., carbohydrates), complex mixtures, and chemical reaction monitoring. In summary, the improvements in the instrumentation have enhanced the reliability and quality of dispersion spectroscopy and enabled further extension of the range of application.

Keywords: mid-infrared spectroscopy; quantum cascade lasers; dispersion spectroscopy; refractive index sensing; Mach-Zehnder interferometer; liquid-phase analysis

Session 7

13th May, 13:30 – 14:30, ICP-MS

27	13:30 – 13:45	**Determination of technology-critical elements (TCEs) in 7 plant reference materials by ICP-MS/MS** Simone Trimmel, Montanuniversität Leoben, Department Allgemeine, Analytische und Physikalische Chemie
28	13:45 – 14:00	**LA-ICP-TOFMS: High-resolution imaging and quantification of platinum in mouse organs and tumor tissue** David Loibnegger, Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna
29	14:00 – 14:15	**Application of Diffusive Gradients in Thin Films (DGT) in Combination with LA-ICP-MS for Tracking Localised Aluminium Corrosion** Gulnaz Mukhametzianova, Montanuniversität Leoben, Chair of General and Analytical Chemistry
30	14:15 – 14:30	**Statistical analysis of the distribution of the cell cycle phases of different cell cultures via LA-ICP-TOFMS**, Claude Molitor, Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna

Statistical analysis of the distribution of the cell cycle phases of different cell cultures via LA-ICP-TOFMS

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The progression of cells via the cell cycle phases is one of the most important processes in tissue homeostasis and it is known that an imbalance in those cell cycle phases plays a major role in disease mechanisms. Such dysregulations in the cell cycle phases play a major role in diseases like cancer, atherosclerosis and inflammations.[1] The analysis of the cell cycle phases by flow cytometry is already routinely used and recent studies also showed the potential of mass cytometry by using metal-tagged antibodies in cell cycle analysis.[2] Although there are already robust methods for the analysis of the four phases, there is still a knowledge gap regarding the effects of different treatments on the distribution of the cell cycle phases. Aim of this work was the statistical analysis of the four cell cycle phases of different cell lines via single cell laser ablation inductively coupled plasma time-of-flight mass spectrometry (LA-ICP-TOFMS) after treatment with different drugs. For the identification of the four phases, the cells were treated with metal labeled antibodies, which tag proteins specific for the different phases whereas the S-phase is labeled by the incorporation of cell ID IdU during the cell cultivation. Additionally, an Ir-intercalator was used, which enables the segmentation of the single cells, which is necessary for statistical single cell analysis. Cytospins were measured by LA-ICP-TOFMS using a laser spot size of 2 µm and oversampling in both directions to reach a (Sub)cellular resolution. This work will enable the differentiation of the cell cycle phases within tumors, tissues or organs and thus bring a deeper understanding of the distribution of for example a drug in tissues according to the cell cycle phase. Because different drugs have different uptakes in different cell cycle phases and also different toxicities. A combination of several drugs show synergistic effects like cisplatin and taxol do for the treatment of human ovarian carcinoma cells in vitro. Taxol herein induces an miotic arrests[3] whereas cisplatin forms DNA-adducts and leads to apoptosis especially in G2 and the M-phase.[4] So this work will constitute a new method to analyze how drugs work and where their limitations and advantages are.

Keywords: LA-ICP-TOFMS; single-cell-analysis, cell cycle phases, laser ablation

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Determination of technology-critical elements (TCEs) in 7 plant reference materials by ICP-MS/MS

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Technology-critical elements (TCEs) are a non-uniformly defined group of elements which are deemed critical for modern technology and our societies due to their limited availability in relation to global demand. Their peculiar chemical properties make them indispensable in the development of high-tech applications across various fields, such as information, telecommunication and energy technology, semiconductors, alloys or catalysts. Their increased use during the last couple of decades can be expected to have resulted in elevated levels in the environment. In the course of the FWF-funded project TecEUS (P 33099-N; www.teceus.at), the occurrence of TCEs in the environment is investigated with a special focus on plant samples from urban greening. The low levels of these elements remain an analytical challenge.

This talk presents an optimized analytical methodology for the determination of 52 elements by ICP-MS/MS: Li, Be, Na, Mg, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Y, Nb, Mo, Ag, Cd, Sb, Te, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Ta, Tl, Pb, Bi, Th, U. Seven plant certified reference materials (NIST SRM1515 Apple Leaves, NIST SRM1547 Peach Leaves, BCR-129 Hay Powder, BCR-670 Aquatic Plant, GBW07603 Bush Twigs and Leaves, GBW10015 Spinach Leaves and NCS ZC73036a Green Tea) were analysed. Closed-vessel microwave digestion was optimized using HNO_3, H_2O_2 and HBF_4. Elements with spectral interferences (e.g. Fe, Ge and REEs) were successfully analysed in mass-shift mode with N_2O as alternative reaction gas. Most elements could be quantified in all 7 CRMs. Compared to conventional techniques involving pre-concentration and matrix-separation, the method is fast, easy to implement and versatile. *LOD*s range from 5.05 fg g^{-1} (^{159}Tb in Spinach Leaves) to 11.3 ng g^{-1} (^{44}Ca in Spinach Leaves). Recoveries are ranging between 80 and 120%. The suggested mass fractions can be used as literature values in future studies.

Keywords: SRM1515, SRM 1547, BCR-129, BCR-670, GBW07603, GBW10015, ZC73036a, N_2O, HBF_4, Microwave digestion;

LA-ICP-TOFMS: High-resolution imaging and quantification of platinum in mouse organs and tumor tissue

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Laser ablation (LA) in combination with inductively coupled plasma-time-of-flight mass spectrometry (ICP-TOFMS) has become a powerful imaging tool to detect endogenous elements as well as metal-based anticancer drugs (Pt, Ru…) or elements (rear earth elements) from metal-labeled antibodies. Therefore, the technique provides promising applications in cancer research, proteomics, metallomics and in the medical sector.

LA-ICP-TOFMS with a resolution down to 1 µm was used to image tumor tissue and organs of colon cancer bearing mice treated with oxaliplatin or the analogous platinum(IV) compound. The platinum distribution in the tissue sections was quantified at the single-cell level using gelatin-based micro-droplet standards spiked with elemental standard solutions [1]. The quantitative platinum uptake was compared between the different treatments and mouse tissue samples.

Furthermore, metal-labeled antibodies were used to stain the tissue sections with functional and structural markers, including markers for DNA damage, proliferation, collagen and alpha smooth muscle actin. The use of metal-conjugated antibodies allowed to characterize the tumor microenvironment and to visualize the platinum uptake in different cell types and regions of interest within the tumor tissue and organ samples. Histological stains of consecutive sections provided additional information on the tissue structure and validated the findings made by LA-ICP-TOFMS imaging.

Keywords: 2D imaging, Laser ablation, ICP-TOFMS, metallomics.

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Application of Diffusive Gradients in Thin Films (DGT) in Combination with LA-ICP-MS for Tracking Localised Aluminium Corrosion

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Passive, non-destructive chemical imaging using diffusive gradients in thin films (DGT) in combination with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has become an important tool to visualize and quantify chemical processes occurring at reactive interfaces *in situ*. While the increasing application of DGT LA-ICP-MS in environmental science substantially improved our knowledge of e.g. biogeochemical cycles in nature, its recent application in materials science opened the door to study metal corrosion processes at unprecedented spatiotemporal resolution.

In this study, we develop and apply DGT LA-ICP-MS for *in situ* tracking of pitting corrosion, a highly localised form of corrosion that initiates the formation of cavities in the metal, which is difficult to detect due to the small size of early pits. The 7075 aluminium (Al) alloy (AA7075), containing Zn (w = 5.6-6.1 %), Mg (w = 2.1-2.5 %), and Cu (w = 1.2-1.6 %), is used as sample material that is being widely applied in aircraft structural parts where susceptibility to pitting corrosion is critical because pits can act as starters for fatigue cracks. Advanced polyacrylamide-based DGT binding gels with a highly homogeneous distribution of Chelex (iminodiacetate) only, or both Chelex and Metsorb (TiO_2) binding phases are evaluated and applied for the simultaneous and quantitative sampling of corrosion products of Al, Mg, Zn, and Cu. The novel approach is combined with optical microscopy to obtain detailed information on surface structural changes during AA7075 corrosion.

Preliminary results of this study show that the application of DGT LA-ICP-MS using Chelex gels enables accurate *in situ* visualization of corrosion reactions on AA7075 exposed to NaCl solution (w = 1.5 %) at pH 4.5 and room temperature with different exposure times. Precise localisation of pitting corrosion remains, however, challenging due to the creation of occluded spaces with stagnant solution flow by the current DGT gel deployment setup, inducing less localised crevice corrosion. First results of the development of a novel experimental DGT setup allowing for non-invasive mapping of pitting events will be presented.

Keywords: Pitting corrosion, Diffusive Gradients in Thin Films, LA-ICP-MS, Chemical imaging, Aluminium

Poster Session

12th May, 16:45 - 17:45

1	**Content and inhibitory potential of wheat amylase-trypsin inhibitors as putative triggers of wheat-related diseases** Lisa Call, University of Natural Resources and Life Sciences, Vienna, Institute of Plant Breeding and Institute of Food Technology
2	**Identification of hypoallergenic soy and wheat cultivars using specific soy and wheat allergens** Andrea Krisai, University of Natural Resources and Life Sciences, Vienna, Austria
3	**Preparation of Immunogens for the Molecular Measurement of Food Allergens with antibody-based Immunoassays** Michael Wiederstein, University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology
4	**Pushing the limits of adsorption enhanced attenuated total reflection spectroscopy using metal-organic frameworks for trace analysis of phosphates in water** Felix Frank, Institute of Chemical Technologies and Analytics, Technische Universität Wien
5	**An Automated Tool for Quality Assessment of Raman Spectra** Daniel Zimmermann, FH Wiener Neustadt Biotech Campus Tulln
6	**Laser Based Mid-IR Spectroscopy for Monitoring Temperature-induced Protein Denaturation of BSA and Stabilization Effects of Sugars** Shilpa Vijayakumar, Institute of Chemical Technologies and Analytics, Technische Universität Wien
7	**Theory, implementation and characterization of low-frequency Laser-Cavity-Locking schemes for Interferometric Cavity-Assisted Photothermal Spectroscopy (ICAPS)** Stefan Lindner, Institute of Chemical Technologies and Analytics, Technische Universität Wien
8	**Mass balance analysis of PFAS in soil, snowmelt and ski waxes** Viktoria Mueller, Institute of Chemistry, University of Graz, Universitätsplatz
9	**A new solvent system for the analysis of hydroxy groups of lignosulfonates by ^{31}P NMR** Gerhild K. Wurzer, University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Chemistry, Institute of Chemistry of Renewable Resources

10	**Volumetric Absorptive Microsampling for drug use confirmation with non-targeted LC-MS/MS** Vera Reinstadler, Institute of Legal Medicine and Core Facility Metabolomics, Medical University of Innsbruck
11	**An Exposome-Scale Sample Clean-up Method based on Solid Phase Extraction** Yunyun GU, Department of Food Chemistry and Toxicology, University of Vienna
12	**Influence of sample preparation on protein identification in bottom-up-proteomics** Theresa Reischenböck, FH Wiener Neustadt – Biotech Campus Tulln
13	**Antarctic psychrotolerant bacteria enhance plant tolerance to cold stress by modulating the plant metabolome** Giorgio Licciardello, Center Agriculture Food Environment, University of Trento, Italy
14	**Development of a negative-thermal gradient GC for fast gas chromatography and its application for the study of volatile products formed in lithium-ion batteries** Bernhard Klampfl, Vienna University of Technology, Institute of Chemical Technologies and Analytics
15	**Novel cellulose-based stationary phases for chiral separations using HPLC** Anna Florentina Lehrhofer, University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Chemistry, Institute of Chemistry of Renewable Resources
16	**Miniaturized LC in small molecule -omics – sensitivity vs. coverage** Veronika Fitz, Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna
17	**Integrating veterinary drugs and pesticides in a targeted LC-MS/MS exposome approach** Md Zakir Hossain, Department of Food Chemistry and Toxicology, University of Vienna
18	**Quick determination of erucic acid in mustard oils and seeds** Bernhard Blank-Landeshammer, University of Applied Science, Wels, Austria;

Content and inhibitory potential of wheat amylase-trypsin inhibitors as putative triggers of wheat-related diseases

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Although wheat is one of the most important crops in the human diet, its consumption can be linked to several adverse reactions, including celiac disease, wheat allergy, and non-celiac wheat sensitivity. In addition to gluten proteins, which are considered to be the dominant triggers of these wheat-related diseases (WRDs), a group of non-gluten wheat proteins called amylase-trypsin inhibitors (ATIs) has been identified to be involved in the clinical pathogenesis of WRDs [1]. As their name implies, ATIs are able to inhibit the enzymatic activity of human and mammal amylase and trypsin leading to incomplete digestion of starch and proteins which can cause gastrointestinal symptoms such as gas production, abdominal pain and bloating [2]. To study this protein class in detail, this study comprises the quantification of ATI contents by RP-HPLC as well as the determination of *in vitro* inhibitory activities in modern wheat (*Triticum aestivum*) samples according to recently published methods [2,3]. In addition, individual samples were blended to form a sample mix that was used to validate the methods. This study revealed that ATI levels and their inhibitory potential against amylase and/or trypsin are not correlated. Consequently, both ATI concentrations and inhibitory activities need to be evaluated to help understand their role in wheat sensitivities.

Keywords: *Triticum aestivum*, wheat sensitivity, enzymatic assay, high-performance liquid chromatography

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Identification of hypoallergenic soy and wheat cultivars using specific soy and wheat allergens

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Exposure to allergens is a major factor determining whether an atopic individual can become sensitized to a given allergen and explains why allergic populations from different parts of the world exhibit different molecular sensitization profiles. It has been recently shown that man-made replanting of landscapes can cause a profound alteration of the profile of allergic sensitization already in two generations of inhabitants suggesting that it is in principle possible to influence allergic sensitization by anthropologic changes of the biome. Wheat and soy represent some of the most important plant foods and represent common allergen sources. Wheat pollen and wheat seeds contain potent inhalant and food allergens, respectively as well as antigens involved in celiac disease (CD) and non-celiac wheat sensitivity (NCWS). This study aims to identify hypoallergenic wheat and soy varieties, which lack or contain lower levels of allergens but preserve their nutritional value and can be easily cultivated. To identify hypoallergenic wheat and soy varieties, different wheat species and soy varieties from collections of genetic resources from Europe will be screened with allergen-specific antibody probes for the presence and amounts of important food allergens and pollen allergens. In addition, a screening of species/varieties and their tissues with IgE antibodies from allergic patients will be performed and the allergenic activity of the species will be tested in basophil activation tests to identify hypoallergenic cultivars.

Keywords: Wheat allergens, Soy allergens, IgE, antibodies, recombinant

Preparation of Immunogens for the Molecular Measurement of Food Allergens with antibody-based Immunoassays

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Food allergy is a world-wide occurring health issue affecting 2-10% of the total population. Considered a pediatric phenomenon, the majority of affected children outgrow their food allergy and develop tolerance. Nevertheless, some food allergies can be persistent and have a major impact on the life quality of allergic patients and might even cause severe allergic reactions such as anaphylaxis. The most common “treatment” of food allergy is allergen avoidance, based on food labelling. Existing labelling regulations do not indicate any threshold levels or applied detection methods, but knowing the actual concentration of allergenic material in food is crucial for allergen avoidance and preventing allergic reactions. Among the big eight food allergy inducing foods is soybean, which is the most used vegetarian protein provider and common ingredient of industrially produced foods due to agricultural, nutritional and industrial benefits. We focus on the development of immunoassays such as enzyme-linked immunosorbent assays (ELISA) or lateral flow devices (LFD) to qualitatively and quantitatively detect soybean allergens in food, based on the availability of soybean allergen-specific antibodies recognizing and binding to the soybean allergen(s). Major soybean allergen targets are Gly m 4 (pathogenesis-related-10-protein), Gly m 5 (beta-conglycinin), Gly m 6 (glycinin) and P34 (cysteine protease). Crucial step of antibody production is the preparation of well-defined immunogens containing the allergen of interest, which are used for the immunization of Balb/c mice. Common approaches to produce immunogens based on the allergen size include cut-off filtration, size-exclusion chromatography (SEC) in combination with high performance liquid chromatography (HPLC) or recombinant allergens, although the use of natural extracts led to better immunological responses of Balb/c mice and higher yields of antibodies. We used these methods to produce well-defined immunogens from crude soy flour, verified by SDS-PAGE and mass spectrometry, to generate soy allergen-specific monoclonal antibodies, identified by ELISA and Western Blot screening. Those antibodies will be applied in highly sensitive and specific immunological assays for measuring soybean allergen exposure in food samples by detecting the real molecular soybean allergen amount, ideally in a personalized manner. These immunoassays will help to facilitate allergen risk management of soybean allergic patients.

Keywords: Food Allergy, Food Allergen Detection, ELISA, SEC-HPLC, monoclonal Antibody

Pushing the limits of adsorption enhanced attenuated total reflection spectroscopy using metal-organic frameworks for trace analysis of phosphates in water

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Phosphate contamination due to agricultural runoff and urban wastewater is one of the main causes of eutrophication and thus loss of water quality. To combat eutrophication caused by the accumulation of phosphates, it is critical to monitor trace amounts of phosphate in water. While conventional mid-infrared (mid-IR) spectroscopy is able to detect phosphate in a complex matrix due to its chemical selectivity, its sensitivity is limited due to the high absorption of water bands impeding the use of long interaction lengths. To combat this, preconcentration schemes combined with evanescent field spectroscopy aim at enriching the analyte into the probed volume.
In this work, we made use of the versatility and defined porosity of the novel material class of metal-organic frameworks (MOFs). The structure of the MOF NH_2-MIL-88B(Fe) is tuned to selectively enrich phosphates from the aqueous phase into the confined pores of a thin MOF film coated onto the surface of an attenuated total reflection (ATR) prism. Using this technique, we present a mid-IR based sensing platform, capable of the detection of sub-ppm traces of phosphate in water without the need of extensive sample preparation. We opted for a concept of easy applicable and removable single-use sensing layers on attenuated total reflection prisms to overcome the struggle of desorbing chemisorbed phosphates, while still guaranteeing robust results. For that, a facile, reproducible workflow for the preparation of thin NH_2-MIL-88B(Fe) layers onto the ATR substrates was developed.
Calibration of the phosphate sensor was performed using aqueous solutions of monobasic phosphate, performing sequential injection analysis to standardise sampling. The sequence included internal referencing of the respective sensing layers to allow for stable results regardless of the applied MOF layer. Calibration curves followed the Langmuir adsorption model, which was used to derive a limit of detection (LOD) of 0.14 mg L^{-1} phosphorus in water.

Keywords: Mid-IR spectroscopy, metal–organic frameworks, sensing, phosphate, trace analysis

An Automated Tool for Quality Assessment of Raman Spectra

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High quality data is the main prerequisite for successful data analysis, as aptly summarized by the phrase “garbage in, garbage out”. In Raman spectroscopy, the first step in ensuring data quality is usually the removal of low-quality spectra. This is especially important in Surface-enhanced Raman Spectroscopy (SERS), where an inhomogeneous distribution of nanoparticles in the sample leads to high variations in spectral quality. Identifying and removing low-quality spectra is however often still a manual process, which is both time-consuming and inherently subjective. An automated and objective quality assessment procedure is therefore needed to enable the use of SERS as a routine analytical method. [1]

For this purpose, we propose an automated tool for estimating the quality of spectra based on the intensity and number of peaks. First, the baseline of each spectrum is estimated and subtracted to remove background fluorescence. Peaks are then identified using a second derivative Savitzky-Golay-Filter, allowing for the detection of low-intensity peaks which would not be recognizable in the original spectra while simultaneously suppressing noise.

The window size of the Savitzky-Golay-Filter and the threshold value for the second derivative control the sensitivity of peak detection and must be carefully selected. If these parameters are set too large, smaller peaks are smoothed out and are not detected. In contrast, values that are set too small lead to an increasing number of false positives.

Finally, the number of peaks and their intensity is used to calculate a quality score. We implemented multiple scoring options which can be selected by the user, depending on whether a higher intensity or a higher number of peaks is considered more important.

To test the tool, we applied it in two different scenarios. During SERS method development, we used the resulting quality scores to compare the suitability of different nanoparticles. The mean quality score of 50 spectra was used to select the highest-scoring SERS method. To assess the reproducibility, we also examined the distribution of intensities and peak counts. [2]

Secondly, we applied the tool as part of a data analysis workflow. [3] Principal component analysis (PCA) showed a significant reduction in the number of outliers compared to the complete dataset. Similarly, the predictive performance of supervised classification models also increased.

Visual inspection of individual spectra shows that our tool accurately reflects the spectral quality, confirming its suitability for use in method development and as part of a data analysis workflow.

Keywords: Raman Spectroscopy, SERS, data analysis, data preprocessing

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Laser Based Mid-IR Spectroscopy for Monitoring Temperature-induced Protein Denaturation of BSA and Stabilization Effects of Sugars

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Infrared (IR) absorption spectroscopy is a powerful analysis tool to study the secondary structure of proteins. The most prominent absorption band of proteins in the mid-IR spectrum is the amide I band (1600-1700 cm–1) which is induced by vibrations of the peptide group and allows evaluating the secondary structure. IR spectroscopy has been widely employed in studying protein denaturation due to its pronounced sensitivity towards the intermolecular b-sheet secondary structure which is associated with protein aggregation. For routinely used FTIR spectrometers path lengths for transmission measurements of proteins in aqueous solution are restricted to <10 μm. These low optical paths considerable impair the robustness of the measurements. To circumvent this challenge, external-cavity quantum cavity lasers (EC-QCL) with broadband spectral coverage and large emission powers were employed as a light sources for laser-based IR transmission setups, thus facilitating longer transmission path lengths and enabling more robust measurements and lower limits of detection.

In this work, a commercially available EC-QCL based mid-IR transmission spectrometer was used to monitor dynamic changes in protein conformation induced by thermal denaturation. For this purpose bovine serum albumin (BSA) was used which is frequently employed as a model protein for biophysical studies. At room temperature BSA displays an a-helical structure, indicated by a maximum of the amide I band at 1656 cm-1. With increasing temperature, the formation of parallel and antiparallel b-sheets takes place that can be followed in the IR spectra by the appearance of two bands at 1619 cm-1 and 1692 cm-1, respectively.

In a first study, the effect of protein concentration was investigated on the transition temperature of thermal denaturation. In these measurements, IR spectra of BSA solution with concentrations between 30 and 90 mg mL-1 were recorded while gradually heated from 25°C to 85°C. Analysis of the progressions of the band heights attributed to a-helix and b-sheets revealed that the transition temperature decreases for higher BSA concentrations.
Furthermore, in a second study the effect of two disaccharides, sucrose and trehalose, and one monosaccharide, mannose, on the transition temperatures of BSA denaturation was investigated. These sugars are possible candidates to be used as stabilization agent in protein formulations. For this investigation, the BSA concentration was kept constant while adding different concentrations of sugars. It was found that the addition of sugar increased the denaturation temperature for all sugars, however with different concentration dependencies.

In conclusion, mid-IR laser-based spectroscopy was successfully employed for studying protein denaturation in simple and more complex solutions up to temperatures of up to 85°C. The established methods and results show promise for the food and medicine industry as a possible way to study the efficiency of different stabilizing agents for proteins.

Theory, implementation and characterization of low-frequency Laser-Cavity-Locking schemes for Interferometric Cavity-Assisted Photothermal Spectroscopy (ICAPS)

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In this work, different schemes for locking a lasers wavelength to an inflection point of the periodic transfer function of a Fabry-Perot cavity is presented. A proper implementation of such an algorithm in terms of long-term stability, noise level and fast reaction to shifts in wavelength of the Cavity Transfer Function (CTF) is crucial and has major influence on the performance of a gas sensor based on Interferometric Cavity-Assisted Photothermal Spectroscopy (ICAPS). The first laser wavelength-cavity-locking technique that is described, is the constant level locking scheme, where the laser wavelength is adjusted to stabilize a given detector level that corresponds to the CTF inflection point. For this technique, the exact wavelength and corresponding CTF value of the CTF inflection point have to be known. A technique, where the inflection point is directly approached, is the 2*f*-wavelength modulation locking scheme. Here, the laser wavelength is modulated with a sinusoidal signal of small amplitude and the detector signal is demodulated at the second harmonic of this modulation frequency. The zero crossing of this quantity marks, for small modulation indices, the inflection point of the underlying CTF. Besides those techniques with known underlying principles, a third technique that is called stochastic locking scheme, was developed. It is based on the fact, that the third moment (skewness) of a stochastic variable is zero, if the values of this variable are symmetric around its center value. This is fulfilled by a set of CTF values with the inflection point being the mean of those values. The only condition that has to be fulfilled is, that the corresponding set of wavelength values is uniformly distributed, what allows more general forms of modulation, besides the well-known sinusoidal modulation. A detailed theoretical description of the developed laser-cavity-locking techniques is given and the performance of those schemes is numerically simulated and experimentally confirmed. As a showcase and application example, results of sensing carbon dioxide in two isotopologues ($^{12}CO_2$ and $^{13}CO_2$) with a quantum cascade laser (QCL) as excitation source for ICAPS are presented.

Keywords: Fabry-Perot-Cavity, Laser Wavelength Locking, Photothermal Spectroscopy

Target analysis of PFAS in different environmental media

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Per and polyfluoroalkyl substances (PFAS) is a collective term of a series of manmade compounds that consist of at least one perfluoroalkyl moiety CnF2n+1. Due to the extreme thermal and chemical stability provided by the C – F bond energy, these compounds were widely produced since the 1950's for both industrial and commercial applications1 as surfactants and polymers producing non-stick cookware, aqueous film–forming foams or water repelling coatings in food packaging and cloths.1 These compounds can be released to the environment, mostly into sewage, from these products. Since biological processes do not destroy them, they remain in the sludge and can accumulate in biota. Organofluorines such as PFOS have been linked to cancer and therefore have been banned by the Stockholm convention since 2009. However, their concentration still increases in the environment, suggesting the presence of precursor compounds. Sewage sludge is often used as a fertiliser on agricultural lands, thus enable the possibility for plant uptake. Intake of contaminated water and foodstuff are considered one of the primary sources for mobile PFAS to be exposed to humans.

Keywords: perfluoroalkyl, polyfluoroalkyl, soil, snow, ski wax

A new solvent system for the analysis of hydroxy groups of lignosulfonates by ^{31}P NMR

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For quantitation of the hydroxy group contents of technical lignins 31P nuclear magnetic resonance spectroscopy (NMR) is the most common method, as it is both well established and highly accurate. Nevertheless, for lignosulfonates, this approach is still limited due to solubility problems in the commonly used solvent systems (e.g. DMF/pyridine). These problems may result from the wide range of different lignosulfonates from various wood sources, the different purification processes as well as the respective pulping conditions used in biorefineries. For modified or fractionated lignosulfonates, the search for a suitable solvent system is even more difficult. In this study, a new and rapid analytical sample preparation approach for quantitative analysis of hydroxy groups of modified and fractionated lignosulfonates has been developed. The implementation of an ionic liquid, namely 1-ethyl-3-methylimidazolium chloride [EMIM]Cl, into the already established DMF/pyridine solvent system resulted in the complete dissolution of previously insoluble samples. The applicability, accuracy, and robustness of the approach involving the novel solvent system were extensively investigated with lignin model compounds and commercial lignosulfonates, including otherwise insoluble real-world lignosulfonate samples. The results were compared with the known DMF/pyridine solvent system. With the new solvent system in hand, a much wider range of various lignosulfonates can now be analysed. In particular, it was possible for the first time to quantify the hydroxy group contents in ammonoxidised lignosulfonates.

Keywords: Lignosulfonates; Hydroxy Group Determination; Ionic Liquid; 31P NMR; Quantitative Analysis

Volumetric Absorptive Microsampling for drug use confirmation with non-targeted LC-MS/MS

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Blood is the most preferrable biological matrix used for forensic toxicological applications. However, the invasive sampling is associated to several disadvantages, including the obligatory involvement of trained medical personnel. To overcome some of the current limitations, microsampling devices that offer minimally invasive, simple, and inexpensive modes of operation are gaining interest. One such microsampling technique is volumetric absorptive microsampling (VAMS). It enables the accurate, reproducible, hematocrit unbiased collection of capillary blood as dried sample on an absorbent polymeric tip. The blood volume typically collected with VAMS ranges 10-30 µL.

In this report, an analytical workflow for forensic drug testing is presented that for the first time ever successfully combines VAMS with a forensic screening workflow comprising of a generic sample processing method, non-targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS), and compound identification via mass spectral library search.

Fitness of the developed workflow for the intended application was demonstrated by method validation, which included determination of limits of identification and process efficiencies. The test set include 48 compounds representing different kinds of compound classes typically observed in forensic examinations (e.g.: drugs, pesticides, and environmental pollutants).

The developed workflow was applied to the analysis of VAMS samples obtained from drug users. Importantly, the samples were collected by the participants themselves at home and transferred to the laboratory by mail. Thirty participants were included in the study. VAMS samples of sufficient quality were provided by 24 participants. Complete sets of health-related data (i.e., information on sex, age, and drug use) were obtained from 20 participants.

The obtained VAMS samples were submitted to non-targeted LC-MS/MS analysis. Data mining produced 2145 annotations representing 177 different compounds. Consumption of 21 out of 33 self-reported drugs was confirmed. In the majority of cases, an additional number of unreported drug compounds were detected. Thus, on average, a participant consumed 2.5 ± 2.4 drugs (Min 0, Max: 8). The most commonly observed illicit drugs were methamphetamine, cannabis, cocaine, and LSD. Another interesting result was the detection of the new psychoactive compounds fluoroethamphetamine and 5-MeO-MiPT.

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Keywords: Forensic drug analysis, volumetric absorptive microsampling, non-targeted LC-MS/MS, new psychoactive substances

An Exposome-Scale Sample Clean-up Method based on Solid Phase Extraction

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Liquid-liquid extraction and protein precipitation are common sample preparation techniques because of their wide coverage of analytes. However, there are disadvantages, including matrix effects and poor sensitivity and reproducibility. Solid phase extraction (SPE) offers an approach at solving these issues, but it is difficult to apply SPE in exposome-scale sample pretreatment because of its high specificity. With development of multifunctional SPE technique, it is possible to apply this technique in broad sample preparation methods. In this study, two kinds of SPE columns, Oasis HLB (Waters Corporation, USA) and homemade mix-mode of primary secondary amine (PSA) and C18 respectively, were tested because of their multiple interactions with analytes with highly diverse chemical properties. There were 95 environmental and food-related toxicants selected as model analytes, including plastic components, perfluorinated alkylated substances, industrial side products and pesticides, endogenous estrogens, phytoestrogens, mycoestrogens, personal care product ingredients and pharmaceuticals, phytotoxins, disinfection by-products, food processing by-product, and air pollutants. Also selected biotransformation products such as sulfates and glucuronides were included. Spiked water samples in a concentration range of 0.25-250 ng/mL were loaded on the different SPE columns. Liquids were separately collected from processes of loading samples, washing columns and elution step. Retention ability and coverage of analytes were estimated and compared. The results showed that more than 90 chemicals were well retained both in HLB and PSA/C18 columns, but there were strong interactions between some acids and the PSA/C18 sorbent, including perfluorinated alkylated substances, and poor retention of 5-hydroxymethyl-2-furanoic acid in HLB cartridges. Further steps of optimization need to be done to improve these challenges and the potential of reducing matrix effects will also be evaluated in future experiments.

Keywords: SPE, exposome, high-throughput.

Influence of sample preparation on protein identification in bottom-up-proteomics

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Tribolium castaneum, the red flour beetle, a widespread pest, is known to be able to survive under extreme conditions, such as very dry environment, low temperatures and even mycotoxin contaminated flour [1]. Furthermore, it serves as genetic model organism and is used for studies regarding insect development, pest biology and comparative genomics [2]. Its robust nature makes it an interesting object to detect possible changes on the proteome level as a reaction to external conditions.

Mass spectrometry has risen to be the method of choice when it comes to protein identification and quantification. In order to obtain a comprehensive picture of the proteome of an organism, cell or tissue sample it is essential to establish a protocol which enables a reliable and reproducible identification of a high number of proteins [3].

A great challenge is the detection of low-abundance proteins in complex protein mixtures [4]. These low-abundance proteins can be of great importance when it comes to changes in the proteome due to treatments or environmental factors.

In our work we focussed on establishing and comparing different methods for protein extraction and sample preparation as a prequel to bottom-up mass spectrometric analysis. Protein samples were subjected to in-solution tryptic digest, measured by nanoLC-MS/MS and quantified using MaxQuant LFQ algorithm [5]. The post-processing was done in R.

The developed method contains a two-dimensional extraction procedure. Various buffers were tested, the first extraction is performed with phosphate buffer, the second extraction step is performed with the remaining pellet using Urea and Triton-X-100 to increase the range of extracted proteins.

The total protein extracts were fractionated using size exclusion chromatography (SEC), in order to reduce ion suppression during the subsequent nanoLC-MS/MS analysis, which led to further increase of total protein identifications.

Keywords: Proteomics, Nano-LC-MS/MS, *Tribolium castaneum*, protein extraction, sample preparation

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Antarctic psychrotolerant bacteria enhance plant tolerance to cold stress by modulating the plant metabolome

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Beneficial microorganisms can protect plants against cold stress, promoting physiological responses of acclimation to low temperatures. Bacteria associated with Antarctic plants are a potential source of beneficial microorganisms adapted to cold environments and the use of these bacteria may represent a sustainable strategy for the protection of crops (e.g. tomato) against cold stress. However, scarce information is available on the molecular mechanisms underlying this process. The aim of this work is to understand the metabolic processes activated by cold-tolerant bacteria on tomato plants and to identify plant metabolites involved in the mitigation of cold stress. We selected four psychrotolerant bacterial strains from the Antarctic plant *Colobanthus quitensis* for their ability to colonize tomato seedlings and to promote plant growth at low temperatures. Surface-disinfected tomato seeds were inoculated with Antarctic bacteria and plant performance under cold stress was compared to mock-inoculated plants. For this, four-week-old plants were exposed to 4°C for seven days. While for characterization of the imposed stress, plants were either sampled and stored immediately after seven days or transferred to temperature conditions of 25°C for another two or four days to allow a recovery from the stress conditions. First measurements showed that bacterium-inoculated plants accumulated higher levels of proline compared to mock-inoculated plants, indicating a high degree of protection from osmotic stress. Additionally, bacterium-inoculated plants showed lower content of malondialdehyde compared to mock-inoculated plants, indicating a lower degree of lipid peroxidation. Currently, comparative LC-HRMS/MS based metabolome analysis of bacterium- and mock-inoculated plants is in progress for known stress related metabolites like polyphenols, amino acids, and sugars. The poster presents first insights into the relative content of phenolic compounds for stressed versus non-stressed plants. The results will help to understand the physiological mechanisms of cold acclimation promoted by Antarctic bacteria in tomato plants.

Keywords: Cold Stress, plant-associated Antarctic bacteria, metabolomics, crop protection.

Development of a negative-thermal gradient GC for fast gas chromatography and its application for the study of volatile products formed in lithium-ion batteries

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Improvements to the longevity, energy-density, cost-efficiency and charging times of lithium-ion batteries are crucial aspects for the viability of this form of energy storage. Work is going on in many laboratories all over the world to improve LIBs by optimizing their electrolyte and establishing a deeper understanding of their degradation and stabilization mechanisms.
To investigate the degradation and aging mechanisms of the electrolyte in-situ, a real-time-(operando) method is required. Identifying and quantifying with high time resolution gas species that are formed during the use of LIBs is crucial to understand the dynamics of the process. To achieve this, a GC/MS system equipped with a fast-measuring system to enable short injection intervals will be employed. Conventional temperature programmed gas chromatography has too long cycle times for this purpose, which prompted us to look for a different approach. Short columns with direct heating allowing for rapid heating rates ($> 400\ K\ min^{-1}$) appear to be a suitable solution. Once that direct heating of the GC capillary is adapted, this opens further new possibilities to improve the separation performance of this system, e.g. by using a thermal gradient in space.
We will report here an experimental setup that has been developed and evaluated to produce a spatially resolved temperature gradient along a GC column. The system can achieve separations of the gaseous homologous series of alkylbenzenes (toluene to pentylbenzene) within under 0.5 mins while maintaining or even improving retention times and resolution in comparison to isothermal measurements respectively.
Use of a negative thermal gradient also is desired to improve the resolution for broad peaks resulting from slow or imperfect sample introduction in a study by Leppert et al.[1]. The results of our experiments show that negative-thermal gradient GC can be a feasible solution for any in-situ or on-line analysis in processes that require a high time resolution.

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Novel cellulose-based stationary phases for chiral separations using HPLC

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Enantiomers are pairs of chiral molecules with geometric structures that are like mirror images of each other. All their physical properties, except for their optical rotation, are identical. However, in a chiral environment, such as in biological systems or during interaction with enzymes, their biological behavior often notably differs. Thus, the two enantiomers have different bioactivities, which is relevant in living organisms, or one of them occurs predominantly (*e.g.* L-amino acids, D-sugars). The selection or separation of one single enantiomer is also crucial for the application of pharmaceuticals, dexibuprofen or esketamine being illustrative examples.

Due to their intrinsic similarity, the two enantiomers can only be separated *e.g.* with advanced chromatographic techniques, such as high-performance liquid chromatography (HPLC) or gas chromatography (GC), provided that the separating materials themselves are chiral (chiral stationary phases, CSPs). Besides using specific synthetic materials, the incorporation of natural chiral molecules into stationary phases is a favorable approach for both the analytical as well as the preparative separation of racemic mixtures into the enantiomers by chromatographic methods. Since Hesse and Hagel first used cellulose triacetate as a CSP in the 1970s, numerous polysaccharide derivatives have been reported *inter alia* by Okamoto *et al.* as suitable chiral selectors for HPLC applications.

In our work, we further exploit the inherent chirality of the biopolymer cellulose for the separation of enantiomers. For an enhanced enantioseparation, cellulose-based derivatives are synthesized and their performance as enantioselectors is tested and evaluated using HPLC after either physical coating or chemical immobilization onto silica as the chromatographic support material. A special focus in this contribution is placed on non-conventional cellulose derivatives, which are synthesized in a bottom-up approach. Cellulose synthesis starting from glucose allows the generation of extremely precise and regioselective functional group patterns in the biomolecule, which might not be accessible by conventional derivatization methods. This way, structure-effect relationships and molecular interactions can be effectively derived. The newly developed cellulose derivatives and CSPs are comprehensively analytically characterized (ATR-FTIR, GPC, liquid- and solid-state NMR spectroscopy, elemental analysis), and tested by HPLC regarding their enantioseparation performance.

Keywords: Cellulose, Chiral stationary phase, Chromatography, Enantioseparation, HPLC

Miniaturized LC in small molecule -*omics* – sensitivity vs. coverage

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Covering a wide spectrum of molecules is essential for exploring the metabolome and exposome in an unbiased manner. While LC-MS based metabolomics and exposomics assays are typically carried out in microbore scale (column i.d. around 2.1 mm), reducing the size of the analytical platform has proven its ability to boost sensitivity for specific -*omics* subfields, especially for application with low available sample amounts. In this study, we elaborate the impact of LC miniaturization on exploratory small molecule LC-MS analysis for samples where sample volume is not a limiting factor, focusing sensitivity, coverage and chromatographic properties essential for non-targeted data analysis. Analysing a panel of endogenous metabolites and relevant environmental contaminants, we assess three flow regimes – analytical, micro- and nano-flow. Miniaturization to micro-flow regime yields moderately increased sensitivity as compared to the nano-setup, where median sensitivity gains of around 80-fold are observed for environmental contaminants spiked to human plasma extract. This gain resulting in higher coverage at low µg/L concentrations is compound dependent. At the same time, the nano-LC-HRMS approach reduces the investigated chemical space as a consequence of the trap-and-elute nano-LC platform. Finally, while all three setups show excellent retention time stabilities, the peak area repeatability of the nano-LC is found to be compromised under rapid gradients. For global metabolomics, micro-LC offers the best compromise between improving signal intensity and physicochemical coverage, despite the fact that only incremental gains can be achieved. Hence, we recommend using micro-LC for wide-target small-molecule trace bioanalysis and global (xeno-)metabolomics of abundant samples.

Keywords: Metabolomics, exposomics, liquid chromatography, high-resolution MS

Integrating veterinary drugs and pesticides in a targeted LC-MS/MS exposome approach

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Human are constantly exposed to a cocktail of chemicals in their body through their diet, the environment and consumer products. Currently, a limited number of chemicals and biomarkers of exposures are monitored in human biomonitoring programs through targeted approach. Unlike sequencing the genome, measuring the chemical exposome i.e. the totality of chemical exposures is a challenging task. LC-MS/MS has been used as a methodology to determine one or several classes of known chemicals in biological and environmental samples. Recently, Jamnik et al. (2022) developed a targeted LC-MS/MS method for simultaneous determination of >80 xenobiotics in human bio-fluids (urine, plasma and breast milk) at the pg-ng/mL level. This method covers highly-diverse xenobiotics including plasticizers, phytoestrogens, mycoestrogens, personal care products, phytotoxins and food processing by-products etc. However, veterinary drugs and pesticides are an important group of xenobiotics that have not been properly included in the method. Therefore, we aim to integrate veterinary drugs and pesticides, which may fill research gaps to better understand the chemical exposome. At this stage, automatic tuning and MRM parameters of veterinary drugs and pesticides has been optimized for 25 compounds. Tuning was performed on QTrap 6500+ mass spectrometer with an ESI source that was operated in positive and negative ionization mode. The aim is to combine and optimize >50 major veterinary drugs and pesticides in this platform and test the method performance for the simultaneous determination of more than 130 xenobiotic chemicals combinedly in human bio-fluid samples collected from different population for exposome-scale biomonitoring.

Keywords: LC-MS/MS, veterinary drugs, pesticides, exposome, human

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Reference: Jamnik T, Flasch M, Braun D, Fareed Y, Wasinger D, Seki D, Berry D, Berger A, Wisgrill L, Warth B (2022). Next-generation biomonitoring of the early-life chemical exposome in neonatal and infant development. ChemRxiv

Quick determination of erucic acid in mustard oils and seeds

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Erucic acid is a naturally occurring 22 carbon acid with double bond in C13 and belongs to the family of *n*-9 (“Omega-9”) fatty acids. It is known to have negative effects on the human health and is therefore regulated in nutritional products such as vegetable oils. In order to determine the content of erucic acid in mustard oil an oil must be pressed or extracted from seeds, the oil derivatized into fatty acid methyl esters and then analyzed by gas chromatography. This process is relatively time-consuming and labor-intensive. Thermally assisted hydrolysis and methylation (THM) is an analytical pyrolysis technique which allows for quick and facile analysis of numerous natural compounds as well as synthetic materials. By using THM we were able to develop a method that can determine the content of erucic acid in mustard oils directly from the mustard seeds, thus avoiding time consuming pressing and off-line derivatization steps. Eleven samples have been tested and the results are in good agreement with conventional oil analysis. It could further be shown that even mustard varieties which are listed as erucic acid free can produce certain amounts of this fatty acid under certain environmental conditions, which supports the need for a fast and reliable screening method which enables analysis directly from the seeds.

Keywords: Thermally assisted hydrolysis and methylation; fatty acids; FAME; edible oils; erucic acid