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Analytical advancements to unravel the effects of organic matter on trace elements cycling in the Environment

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Trace elements play an important role in human health and the functioning of ecosystems. While certain trace elements, such as mercury or arsenic, can be highly toxic, others like selenium or zinc are essential micronutrients for humans, animals, plants, and microorganisms. Assessing and predicting the risks associated with toxicity or deficiency require a comprehensive understanding of the distribution of trace elements in the environment, their transfer between environmental compartments as well as their entry into the food chain.

One well-established fact is that natural organic matter, a main component of terrestrial, aquatic, and atmospheric systems, strongly influences the biogeochemical cycling of trace elements. Unraveling the effects of organic matter on trace element cycling remains, however, challenging due to the intricate nature of organic matter and the low concentrations of trace elements that are furthermore distributed among multiple organic and/or inorganic species, making the detection of individual chemical species difficult.

This talk will present analytical methods based on chromatography coupled with mass spectrometry, which were developed to characterize the molecular composition of organic matter or the associations between organic matter and trace elements. Case studies will also be presented to showcase the insights gained into trace elements biogeochemistry through the application of these methods to lab and/or field experiments.

How to Overcome Analytical Challenges Commonly Encountered in the Analysis of Cr and Cr(VI) in Environmental and Biological Matrices

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Chromium (Cr) mainly exists in the environment as trivalent Cr(III) and hexavalent chromium Cr(VI). Cr(III) is an important micronutrient, while Cr(VI) is an occupational lung carcinogen. The chemistry of Cr plays a major role in its cellular entry and toxic effects. A sensitive and robust method for the simultaneous determination of Cr(III) and Cr(VI) has been developed. The method uses a hyphenated micro liquid chromatography system coupled to inductively coupled plasma mass spectrometry (µLC-ICP-MS) [1]. The method incorporates an EDTA complexation step to stabilise Cr(III). The pH is adjusted to stabilize Cr(VI). Separation was achieved using an anion exchange micro-sized column. This presentation will highlight the analytical challenges (including pH dependency, contamination and soot deposit in ICP-MS) encountered during method development. The method has been applied to environmental and biological samples collected within a European human biomonitoring study [2]. The study aimed to harmonize procedures for human biomonitoring. Human biomonitoring indicates exposure to chemicals by measuring either chemicals or markers of subsequent health effects in body fluids or tissues. This presentation will highlight the harmonization challenges (including interlaboratory comparison and availability of certified reference materials [CRM]). The human biomonitoring study evaluated the occupational exposure to Cr(VI). Samples were collected from 299 workers and 103 controls. The principal biomarker used for biomonitoring of Cr(VI) exposure at the workplace is total amount of Cr in urine. The main limitation of this biomarker is that it is not specific for Cr(VI) since it reflects exposure to both Cr(III) and Cr(VI). We studied the use of potential more specific biomarkers, such as Cr in red blood cells (RBC) and Cr(VI) in exhaled breath condensate (EBC). Cr in RBC reflects the exposure specifically to Cr(VI) since only Cr(VI) is able to pass though the red cell membrane. Cr(VI) in EBC can give specific information on the Cr(VI) levels in the lungs (main target tissue). This presentation will highlight the main findings of this study related to the analytical challenges (including low levels and stability). As indicated in this study, the analysis of Cr or Cr(VI) in environmental and biological samples is subject to challenges. Precautionary procedures needs to be taken during method development, analysis, sampling and storage. For the future success of chromium speciation in EBC, CRMs in water or EBC needs to be made available.

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All Covalently Bound Ion-Selective Membranes for Increased Stability in Potentiometric Sensing

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Solid-contact ion-selective electrodes have gained significant interest over the last decade due to their ease-of-use, miniaturisation possibilities and low maintenance. They can now be routinely found in the bioanalytical field where they are used to measure a wide range of blood electrolytes or in environmental monitoring where they enable the continuous measurement of a large range of relevant ions, such as nitrate, pH or carbonate. Solid contact ion-selective electrodes include an electron conducting material, such as glassy carbon or gold, covered by a transducing material that is known to improve the stability of the signal and suppress undesired ion transport. The last component is a polyvinyl chloride (PVC)-based plasticised membrane loaded with ion-exchanger and ionophore that enable the selective and sensitive sensing of the target analyte. Unfortunately, this system suffers from leaching of membrane components that over time causes drift and loss of sensitivity[1]. To minimise components leaching, different strategies were envisioned such as enhancement of lipophilicity[2] or covalent binding[3]. Increasing the lipophilicity only slows down the leaching and can also be detrimental in terms of synthetic modification and solubility in organic media. The second approach was based on a plasticiser-free cross-linked poly(decyl methacrylate) matrix that was functional if a single membrane component (either ion-exchanger or ionophore) was covalently attached. Although some studies on the topic exist, reports of attempting the covalent linking of all membrane components[4-5] are scarce.

We present here a new strategy for creating a leak-free ion-selective plasticised membrane, where we decided here to take advantage of “Click” chemistry to safely anchor membrane components. Chlorine groups naturally present on PVC can be easily replaced by azide groups, thus generating an ideal platform to perform a “Click” reaction, also known as azide alkyne cycloaddition. Membrane components can in a second step be modified to include an alkyne group, needed for the final covalent attachment. Taking advantage of the high yield of “Click” reactions, alkyne-modified membrane components can be covalently attached in a quantitative manner by controlling the stoichiometry to prevent any leaching. The new electrodes will be tested using thin-layer membranes[6] to accelerate the leaching process and confirm their improved performances compared to conventional membrane that only rely on lipophilicity.

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Rapid Pesticide Screening by Swab Spray Mass Spectrometry

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Ambient ionizations techniques have gained increased interest for rapid qualitative analysis of compounds in the various fields of life sciences and forensics. Analytes are ionized from solutions or surfaces under ambient conditions without any need for sample preparation. Swab spray mass spectrometry is an electrospray-based ambient ionization method that features easy and effective sample collection on surfaces and direct ion generation by application of high voltage and solvent flow to the swab head [1]. Its capabilities have been demonstrated in numerous applications [2].

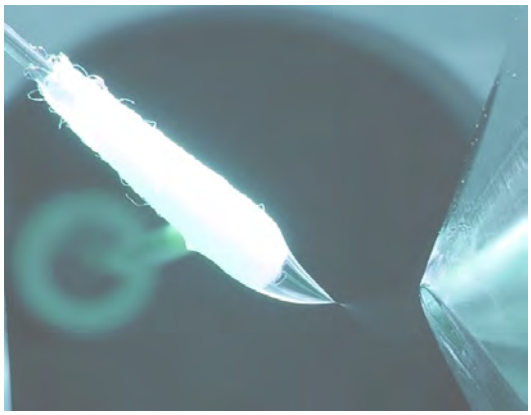

Figure1. Formation of a Taylor cone on the swab head is visible after application of solvent flow and high voltage.

In agriculture a large variety of pesticides are used in vast quantities. The established LC-MS/MS based methods for monitoring pesticide residues on food products require time-consuming sample preparation steps. Ambient ionization methods offer the opportunity for a rapid and hassle-free workflow providing qualitative information about pesticide contamination on food products.
A custom-made swab spray ionization source attached to an Orbitrap Velos mass spectrometer was employed for rapid pesticide screening. The ionization potential is directly applied on the conductive swab handle and the solvent is delivered to the rayon swab head through a capillary. Application of the high voltage induces the formation of a Taylor cone and a jet region which breaks up into the spray plume, where the ionization process takes place. The effect of source parameters and solvents is evaluated with regard to background signals and sensitivity. This setup enables fast detection of pesticides sampled from fruits and vegetables, and their structural confirmation by collision activated dissociation. Additionally, aspects of quantitation are discussed, including the influence of sample surface area and texture on analyte collection, analyte extraction from swab, suppression effects, sensitivity, and reproducibility of the method.

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Targeted phosphoproteomics to reveal mTOR pathway signalling dynamics in zebrafish

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Cells employ reversible protein phosphorylation as a means to transmit information in response to external factors like nutrient levels or stress. Monitoring the activity of molecular signalling networks holds potential for predictive ecotoxicology, for example in assessing the effects of chronic chemical exposure on fish growth. To overcome the limitations of traditional antibody-based methods, we sought to develop a targeted mass spectrometry-based (phospho)proteomics approach to study phosphorylation-based signalling networks. This novel approach enables the simultaneous quantification of phosphorylation and abundance for multiple protein targets, thus eliminating the need to develop and utilize multiple antibodies, which may be lacking for non-mammalian species. We used the zebrafish (*Danio rerio*) embryonic cell line PAC2 as a model, and focused on the mTOR pathway, a central signalling pathway involved in the regulation of cell growth and proliferation. The developed workflow involves a fast cell lysis using 5% SDS for 1 minute, followed by in-column protein trapping and digestion using S-Trap^{TM}. Heavy-labelled synthetic peptides of the endogenous peptide targets are added after the digestion step to determine the recovery after desalting and subsequent enrichment of the phosphopeptides. This allowed us to compare the peptide recovery in the bound and unbound fractions of the enrichment step, for 48 phosphopeptide targets and two enrichment methods. The average recovery was found to be only 14% for TiO_2-beads, but reached 87 ± 9% with Fe^{3+}-NTA beads. Only the latter enrichment method effectively removed interferences and thus improved the detection sensitivity for the targeted phosphopeptides via multiple reaction monitoring (MRM). We applied our method to investigate the mTOR pathway phosphorylation dynamics in the PAC2 cells (i) at different growth stages in cell culture, (ii) after nutrient deprivation, and (iii) after exposure to chemicals, including mTOR inhibitors and chemicals known to impact fish growth *in vivo*. Time-resolved analysis of protein phosphorylation responses within the zebrafish mTOR pathway provides insights on various checkpoints associated with the regulation of cell growth and proliferation.

ALPINAC - A non-target screening algorithm for high'resolution mass spectra and its application to the detection of halogenated greenhouse gases

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Efficient and automated screening of gaseous or liquid samples to detect novel compounds based on their mass spectral fingerprints (non-target screening) is an ongoing computational challenge that goes beyond standard library-based approaches. We present a novel algorithm that uses combinatorial and directed graph methods, taking into account chemical rules, to automatically assign high-resolution mass spectral peaks from gas-chromatography-separated time-of-flight mass-spectroscopy (GC-TOF MS) measurements to possible chemical formulas by considering possible fragmentation pathways. In a further step, this information is used to reconstruct the chemical formula of likely molecular parent ions. We show how this technique can be used to detect unknown contaminants in pre-concentrated air samples and how the algorithm can be extended to reconstruct not only the molecular formula but also the chemical structure of the parent ion.

In search of accuracy: The road to high-quality certified reference materials to achieve comparability of chemical measurements

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The proper analysis of a product is the key step to guarantee high quality and safety for consumers in fields such as healthcare, food and environmental monitoring.

The ability to trace measurement results of an instrument or system ultimately back to a SI unit (Système international d'unités) is a critical aspect of ensuring the accuracy and reliability of measurements. Together with measurement uncertainty, metrological traceability is an important factor for a laboratory to meet the requirements of the ISO/IEC 17025 standard.

Recently, our R&D teams developed ready-to-use certified reference material (CRM) mixtures suitable for use as calibrants in LC-MS or GC-MS methods. All components of the mixes were internally synthesized or sourced externally, individually characterized, and their contents determined by quantitative nuclear magnetic resonance (qNMR) spectroscopy according to ISO/IEC 17025 accreditation [1]. Subsequently, these well-characterized batches were used as raw materials to prepare the final solutions by gravimetrically dissolving and diluting them in an appropriate solvent according to the ISO 17034 accreditation workflow [2]. The resulting bulk solutions were collected in ampules and the process controlled through homogeneity testing by LC-IDMS or GC-IDMS, respectively. The final ampules were further investigated thoroughly to guarantee stability of the product during transportation and throughout the whole shelf-life at storage temperature.

This presentation will provide an overview of the above-mentioned process along with a discussion of the importance for maintaining metrological traceability and reporting of overall uncertainty. Examples will be provided to demonstrate the wide-ranging application scope of certified reference materials in analytical testing contexts.

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[2] ISO 17034:2016, “General requirements for the competence of reference material producers”

Identifying Biomarkers and Habitability Indicators on Polygonal Structures using Laser Mass Spectrometry

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The search for signatures of extra-terrestrial life, past or present, has been a fundamental driving force for space science. Recently, the search has been promoted by measurements of the Martian surface from several space missions, having led to an increase in the understanding of Mars and its environmental history. Yet, the question of the ideal exploration site for the search of life remains unanswered. The current missions focus on formerly subaqueous environments, such as deltaic structure and water bodies [1]. These exploration sites are capable of burying organic matter, due to the typical rapid sedimentation; however, the biosignature preservation in those high-energy settings is often compromised by oxidizing fluids and gases [2]. In contrast, quiescent settings, such as lakes, are more suited to preserve biomarkers. As many of these ancient water bodies were saline, they formed salt deposits when they dried out and the dissolved salt precipitated. During precipitation, biomarkers can be buried and thereby shielded from the harsh environment, such as the radiation. On the surface of Mars, significant amounts of salt deposits have been identified, displaying prominent surface features such as a polygonal structure, visible from orbit (e.g. HiRISE imaging). The same polygon structures are also found in salt depositions on Earth, like in Atacama desert or in the Boulby Mine, UK, both Mars analogue sites. This contribution presents results of a recently performed study on those polygonal structures found in the halite of the Boulby Mine [3]. The measurements were conducted using a space-prototype laser ablation ionisation mass spectrometer (LIMS) designed at the University of Bern for *in situ* space research [4]. The LIMS instrument consists of a miniature reflectron-type time-of-flight mass analyser (160 mm x Ø 60 mm) and a femtosecond pulsed laser system (wavelength λ = 258 nm, laser pulse width of τ ~190 fs, pulse repetition rate of 1 kHz) for the ablation and ionisation of solid material [5]. The polygons contain two optically distinct features: the light interior and the dark edges, for both of which, the chemical composition was determined and compared. A special emphasis was put on the comparison of CHNOPS element abundances, as they serve as biomarkers. The recorded data show that the edges of polygonal structured salt deposits are preferential sites for element accumulation, with a significant increase in CHNOPS elements and other minor and trace elements necessary for the formation and maintenance of life. Therefore, such polygonal structures are promising sites for the detection of signatures of life for future *in situ* space exploration missions. The measurement procedure and results will be discussed in regard to possible future missions for the search of life on Mars.

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Detection of Nucleobases and other N-Heterocycles with Laser Desorption Ionisation Mass Spectrometry for In Situ Space Exploration

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Humanity has long been wondering about the origin of life and the possible existence of extra-terrestrial life. Lately, in situ detection of molecules present on other planetary objects in our solar system has become feasible by means of spacecraft landing and the applying novel measurement technologies. This facilitates future missions with an astrobiological focus to identify the possible presence of prebiotic molecules and molecular biosignatures that could provide us with information about the origin and presence of life.
Several groups of molecular compounds (both biotic and abiotic) are of astrobiological interest to be identified on planetary surfaces by space exploration missions [1,2]. Examples are amino acids, polycyclic aromatic hydrocarbons, sugars, lipids, and N-heterocycles. Nucleobases, which are N-heterocycles, are monomeric units of RNA and DNA as well as cofactors of several enzymes in present-day life on Earth [3]. Multiple N-heterocycles, including nucleobases, have been identified in meteorites and some are thought to be directly involved in primitive biological systems [4].

Instrumentation for the reliable detection of these molecules in space research is severely hampered compared to laboratory-sized instruments, due to restrictions on size, weight, and energy consumption. Moreover, instruments have to be robust, as well as have high sensitivity and broad dynamic range coverage to detect trace abundances. A lightweight and robust instrument, which complies with the requirements for space instrumentation, was designed at the University of Bern for the detection of bio-relevant molecules for future space exploration missions [5]. Organics Information Gathering Instrument (ORIGIN) is a space prototype Laser Ionisation Mass Spectrometer (LIMS) operated in laser desorption mode. The system consists of a ns-pulsed laser system for the desorption and ionisation of molecules and a compact reflectron-type time-of-flight (R-TOF) mass analyser (160 mm x Ø 60 mm) for the separation of ions [6].
Current measurement capabilities of ORIGIN were investigated regarding biosignature detection, as well as the laser desorption conditions [5,7,8]. In our contribution, the ORIGIN measurement procedures and setup will be discussed in detail. We will show results on the feasibility of the detection and identification of nucleobases and other N-heterocycles using ORIGIN. In addition, we will discuss the sensitivity and dynamic range of the instrument and the influence of the sample substrate on the measurement performance of ORIGIN.

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Single-Molecule Peptide Identification using Fluorescence Blinking Fingerprints

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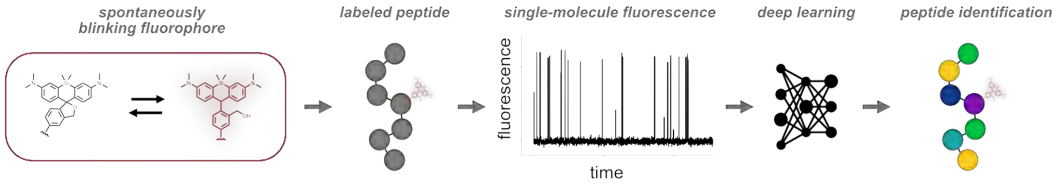
The analysis of the proteome is complicated by the presence of isoforms, post-translational modification (PTM), and the insufficient correlation between the abundance of protein and the transcriptomic or genomic information. As the current state-of-the-art, mass spectrometry-based proteomics methods remain limited in their sensitivity and dynamic range compared to the established single-molecule approaches in genomics and transcriptomics.[1,2] In particular, single-molecule identification of peptides and proteins would enable the analysis of biomarkers that are present in very small quantities, for example in diluted clinical samples, single cells, or isolated organelles.[3]



In this work, we provide a proof-of-concept of a fundamentally new approach to identify single peptide molecules, including subtle PTMs, that does not rely on sequencing.[4] Although this method is presently constrained to targeted studies, it holds potential as a widely applicable, rapid, and accurate single-molecule proteomics technology. Our approach exploits the emission of a spontaneously blinking fluorophore to capture information on the chemical environment of the probe.[5] We use single-molecule fluorescence measurements and a deep learning model based on convolutional, gated-recurrent unit layers to identify the peptide of interest in a targeted manner. By implementing Monte Carlo dropout, we obtain an uncertainty measure for classification which we use for filtering out low-quality traces. We first validate our method on a small set of unnatural peptides of the same length but varying amino acid sequences, which allows us to classify single peptide molecules with overall accuracies > 90 %.[4] Furthermore, the method can be applied to differentiate between peptides with a variable number and position of PTMs. The applicability of the analysis is demonstrated for two PTMs, including the epimerization of an amino acid which would be very difficult to analyze by mass spectrometry. We envision that the technology is adaptable to molecules beyond peptides and proteins depending on available conjugation strategies. Moreover, the method has the potential for further refinement in experimental and analysis aspects to improve the accuracy and extend the applicability to real biological samples.

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Droplet-based microfluidic platform for operando X-ray absorption spectroscopy of single-atom heterogeneous catalysts in organic synthesis

T. Moragues[1], G. Giannakakis[1], C. Borca[2], T. Huthwelker[2], A. Bugaev[2], A. Clark[2], A. deMello[1], J. Pérez-Ramírez[1]*, S. Mitchell[1]*

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Single-atom heterogeneous catalysts (SACs), bridging the gap between the homogeneous and heterogeneous approaches, have recently emerged as a promising alternative for catalyzing organic reactions.[1] That said, very little knowledge exists about the catalytic cycle of reactions on supported catalysts. This is because unlike organometallic catalysts, for which a plethora of characterization techniques is employed to gain a better understanding of the catalytic cycle, *in-situ* and operando tools for heterogeneous catalysts, including SACs, are limited. As such, mechanistic insights largely rely on density functional theory (DFT) calculations and still lack experimental validation.[2] This study introduces a droplet-based microfluidic platform suitable for operando X-ray absorption spectroscopy (XAS) of SACs. The strategy relies on safely flowing catalyst suspensions of precisely tuned size in droplet-based microfluidic systems (**Fig. 1a**).[3] The encapsulation of the particles within the droplets, which act as isolated reactor vessels, prevents clogging and enables time-resolved operando measurements. In this study, the Pd L_3-edge and K-edge of palladium on carbon nitride (Pd/ECN) SACs are investigated under Suzuki-Miyaura reaction conditions (**Fig. 1b**). Notably, we demonstrate the importance of performing *in-situ* XAS by evidencing the differences in Pd L_3-edge X-ray absorption spectroscopy near edge structure (XANES) obtained during operando and ex-situ measurements (**Fig. 1c**). Most importantly, we lay a vital foundation for advancing the mechanistical understanding of liquid-phase organic syntheses catalyzed by SACs.

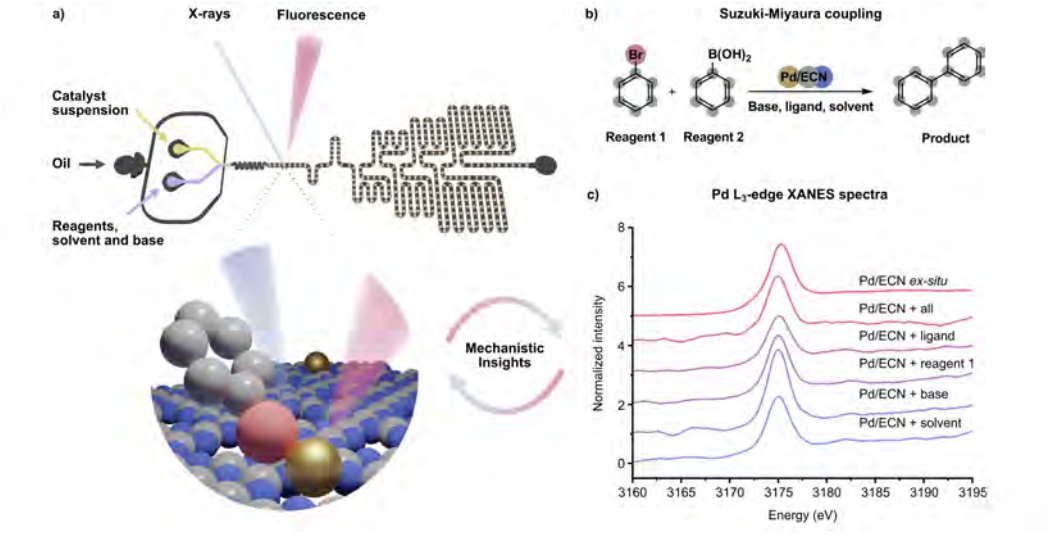

Fig. 1a Schematic of the channel pattern and operation principle in the droplet-based microfluidic device. Time-resolved XAS measurements can be acquired for increasing residence times. **b** Schematic of the studied Suzuki-Miyaura coupling catalyzed by Pd/ECN heterogeneous SAC. **c** Pd L_3-edge XANES spectra acquired in the microfluidic device for various reaction conditions.

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Can breath components be quantified with secondary-electrospray ionization coupled to mass spectrometry?

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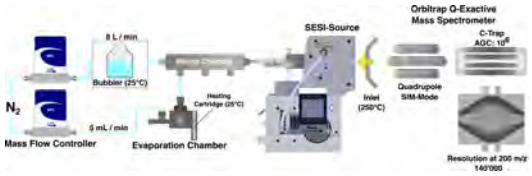
Absolute quantification is desirable for any analytical technique, especially in the context of medical diagnosis. As an alternative to traditional blood analysis, on-line breath analysis using secondary-electrospray ionization (SESI) coupled to mass spectrometry (MS) could serve as a non-invasive diagnostics tool. To reach the full potential of this technique, a gas standard generation system based on the controlled evaporation of liquid analytes and their dilution in a carrier gas stream was developed to produce low-concentration (down to part-per-trillion) standards.[1] This system can operate at the same flow rates as typical human exhaled breath and under humid conditions. To test the analytical capabilities of the system, short-chain fatty acids were used to test the limits of detection and quantification, as well as the linearity. To elucidate whether this system could be used to calibrate a SESI-MS system externally, ion suppression effects also had to be characterized. Ion suppression was postulated to be potentially present in SESI[2], which was investigated using the gas generation system described above. It was shown that ion suppression in SESI is mainly a gas-phase phenomenon. For this purpose, gas standards of D_6-acetone, D_3-acetic acid and pyridine were generated and their impact on each other's signal was determined. D_3-acetic acid seemed to be most affected by rising levels of D_6-acetone and pyridine, whereas pyridine was the least affected by increasing the concentrations of the other two compounds. This indicated a mechanistic rationale for signal suppression within SESI related to gas-phase acid-base chemistry. Pyridine with the highest gas-phase basicity was not affected by increasing concentrations of D_3-acetic acid and D_6-acetone, while the other two compounds that have lower basicity are more strongly affected when the concentration of pyridine was increased. Regarding quantification of breath metabolites with SESI-MS, the use of internal standards is advised.



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Optimal sensitivity regime for ^{1}H detected relayed DNP

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Dynamic Nuclear Polarization (DNP) is now a well-established hyperpolarization technique that can significantly enhance the sensitivity of magic angle spinning (MAS) solid-state NMR. There has been a lot of interest in combining DNP with high magnetic field and fast MAS to benefit from the significantly improved ^{1}H resolution and high sensitivity and thus allow the implementation of ^{1}H-detected 2D correlation schemes. Recently, demonstrating this, we reported the first DNP MAS experiments at 21.2 T using a 0.7 mm MAS probe that enabled spinning rates of up to 65 kHz, at ~100 K. [1] However, under these conditions, despite the high enhancements observed in the radical solution, in the context of relayed DNP (R-DNP), faster MAS rates have a detrimental effect on the DNP enhancement. This raises the question of whether this decrease in DNP enhancement is also reflected in the sensitivity of the microwave ON experiment. Here we analyze the effect of faster MAS rates on the absolute sensitivity of the experiment recorded under microwave irradiation.

^{1}H-^{1}H spin diffusion is a central component of the hyperpolarization mechanism in MAS DNP, [2] as it conveys hyperpolarization to the bulk of impregnated materials. While faster MAS rates increase ^{1}H sensitivity and resolution, they also reduce the level of hyperpolarization in the polarized materials by reducing the spin diffusion rate. Thus, between these counteracting effects, we predict that there should be an optimal sensitivity regime. Here we perform R-DNP experiments at fast MAS rates using 0.7 mm diameter rotors at 21.2 T to study the trend of the overall sensitivity as a function of MAS rate. We find that at faster MAS rates, the sensitivity gain due to ^{1}H detection overcomes the loss of overall polarization in impregnated materials due to slower spin diffusion.

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Spectroelectrochemical Investigation of the Nitrogenase-like Dark Operative Protochlorophyllide Oxidoreductase (DPOR)

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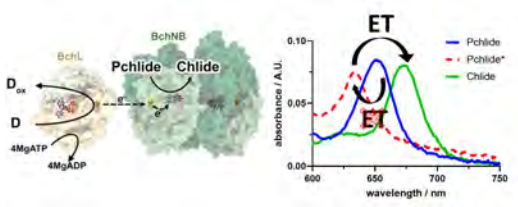
Dark-Operative Protochlorophyllide Oxidoreductase (DPOR) is an enzyme made of a homodimer called BchL and an $\alpha_2\beta_2$ heterotetramer (two catalytic αβ halves) called BchNB. BchL has two ATP binding sites and contains an iron-sulfur cluster, while BchNB has an iron-sulfur cluster and a substrate binding site in each αβ half. This enzyme participates in the biosynthetic pathway of bacteriochlorophyll, enabling photosynthesis by photosynthetic bacteria: the transfer of electrons (which happens through the iron-sulfur clusters) by DPOR's transiently associating proteins is coupled to the hydrolysis of ATP, resulting in the stereoselective $2e^-$ reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide). The substrate reduction can be observed *in situ* by using UV/visible spectroscopy. DPOR has structural and mechanistic similarities with the N_2-fixing enzyme nitrogenase and, as is the case for nitrogenases, neither the dependence of this enzyme on ATP hydrolysis nor the order of events are fully understood. Further, the reduction potentials of DPOR's iron-sulfur clusters have not been reported yet. Thus, spectroelectrochemistry is proposed to be a useful tool to (i) determine these reduction potentials and (ii) study the reduction of Pchlide by DPOR.

In our previous work[1], we proposed three alternative electron donors that support Pchlide reduction in the DPOR system, which can also be used as mediators for electrochemical studies. Now, we use mediated electron transfer to control the electron delivery and thus, the reduction of Pchlide. We investigated the properties of the iron-sulfur clusters in DPOR using spectroelectrochemistry. Moreover, we are able to isolate the ES complex formed when Pchlide is bound to BchNB, (Pchlide*). The study of the binding event enables us to determinate the rate determining step of the reaction and to confirm that ATP hydrolysis does not have a role in the substrate binding step. We propose that the rate-limiting step is the formation of Pchlide* prior to subsequent interactions with BchL. Our research on DPOR is expected to be informative to related metalloenzymes, such as nitrogenase



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N-doping Graphene at Ambient Conditions with N_2-DBD-Plasma and the role of neutral species

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N-doped graphene is a promising material for the oxygen reduction reaction, battery electrodes, and more. Plasma-based methods are an excellent alternative for doping graphene with nitrogen atoms because it allows for post-production treatment, localized doping, and is possible at atmospheric pressure conditions. However, the type of reactive species generated by the plasma and resulting N-doping are not well understood. We doped nitrogen into monolayer graphene by bombarding it with reactive nitrogen species from a low-temperature plasma based on an atmospheric pressure dielectric barrier discharge. After 30 s bombardment, the graphene monolayer on copper had a moderate degree of damage I_D/I_G=1.2 and increase in N-atomand O-atom (content. N-atoms bound as pyrrolic nitrogen (N1s 400.0 eV), while it was impossible to exclude oxygen at atmospheric pressure, which formed a mixture of oxygen-containing functional groups on the graphene. At long treatment times (20 min), the treated area increased radially and the 2D-structure of graphene was destroyed. A part of the increase in N- and O-content was due to adsorbed hydrocarbons, as transfer to XPS in open-air led to an increase in the integrated C1s peak.

N-atom content increased with increasing operating voltage of the DBD source. The N-atom content and type remained unchanged when only neutral reactive nitrogen species bombarded the surface. We hypothesize, therefore, that the primary reactive species resulting in pyrrolic N-doping from the DBD are neutrals such as N(^{4}S) and possibly N(^{2}P).

Oxygen Isotope Analysis of Phosphate by Electrospray Orbitrap Mass Spectrometry for Assessing the Microbial Metabolism in the Environment

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Understanding the impact of human activities on the metabolic state of soil and aquatic environments is of paramount importance to implement measures for maintaining ecosystem services and sustainable access to food, water, and energy. In this project, we explore variations of natural abundance oxygen isotope ratios in phosphate as a new proxy for the holistic assessment of metabolic activity. Given the crucial importance of phosphoryl transfer reactions in fundamental biological processes, we hypothesise that changes in natural abundance $^{18}O/^{16}O$ ratios in phosphate also reflect shifts in the metabolic state of the environmental microbiome as a response to anthropogenic impact.

To make such oxygen isotope ratio measurements available for metabolic studies, we evaluated the applicability of recently introduced instrumental approaches of oxyanion isotope analysis by electrospray ionisation (ESI) Orbitrap high-resolution mass spectrometry for phosphate. To that end, we characterised important ionisation and Orbitrap parameters for precise and accurate $^{18}O/^{16}O$ ratio measurements of phosphate in solutions of variable pH and different sulfate and methanol concentrations through automated flow injection analyses. Based on optimised instrument parameters, we subsequently tested different protocols for the extraction of orthophosphate from aqueous and biological matrices into the methanolic solutions used for quantification of $^{18}O/^{16}O$ ratio in phosphate from environmental samples.

Our data suggest that the ESI-Orbitrap approach to measure oxygen isotope ratios in phosphate could simplify sample preparation and thus increase sample throughput, thereby providing the procedures for in-depth studies of changes in the microbial phosphorus metabolism in the environment.

Nanoscale chemical analysis of Sb_2Se_3 solar cell using tip-enhanced Raman spectroscopy

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class="Standard1">Among second-generation solar cells, Sb_2Se_3-based cells have gained great interest due to their lack of toxicity, higher abundance of Sb_2Se_3 compared to the conventional absorber materials, and highest reported power conversion efficiency (PCE) of 9.2%.[1] The Sb_2Se_3 solar cell is doped with copper and consists of a layered structure, which is shown in Figure 1a. The chemistry and structure of the interface between the layers plays a key role in determining efficiency. At the layer interfaces, chemical defects such as newly formed compounds or structural defects such as inhomogeneous layer separation can critically influence PCE.

Since the interfacial regions in these solar cells have nanoscopic dimensions, their analysis requires a nanoanalytical tool because when using conventional analytical techniques such as confocal Raman spectroscopy, the information from the individual layers averages out, precluding nanoscale analysis. In this study, we demonstrate that tip-enhanced Raman spectroscopy (TERS)[2] is a sensitive analytical tool for non-destructive nanoscale chemical characterization of interfacial regions in solar cells under ambient conditions.

By investigating a Sb_2Se_3 thin film solar cell, we show that TERS imaging can be used to probe this type of solar cell with a spatial resolution of up to 10 nm (Figure 1b). Interestingly, the two layers (Sb_2Se_3 and CdS) do not appear completely separated, as some areas in the Sb_2Se_3 layer show a strong CdS signal (blue pixels in Figure 1b). In addition, high-resolution TERS imaging revealed some regions with bands, which could not be assigned to either Sb_2Se_3 or CdS, indicating the presence of some unknown defects/impurities in these layers.

In summary, this study demonstrates the potential of hyperspectral TERS imaging to investigate defects/inhomogeneities in the interfacial regions of novel solar cells with nanoscale resolution.

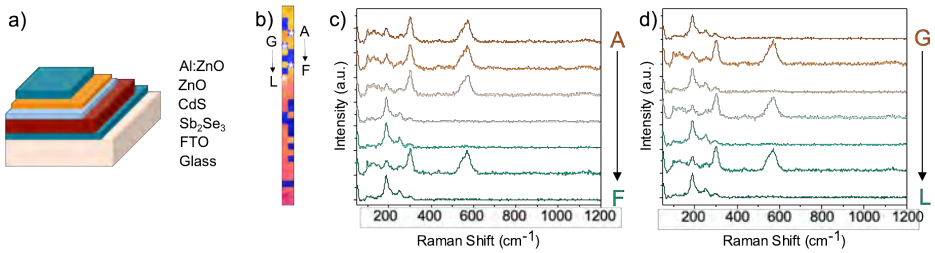

Figure 1 a) Scheme of the layer structure of a Sb_2Se_3 solar cell. b) Overlay of the Raman images generated by the intensity of the CdS Raman band (300 cm^{-1}, blue) and Sb_2Se_3 Raman band (190 cm^{-1}, red) and the oxidized Sb_2Se_3 Raman band (250 cm^{-1}, green). Size: 640 × 20 nm^2. c) Raman spectra were measured along the black arrow A-F marked in Panel b. d) Raman spectra were measured along the black arrow G-L marked in Panel a. All spectra were background subtracted and smoothened. Laser power: 263 µW. Acquisition time: 5 sec. Step size: 10 nm.

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Broadband APT (BAPT): a Versatile APT Experiment with Improved *J*-Compensation and Optimal Suppression of Artifacts in C_q-only Spectra

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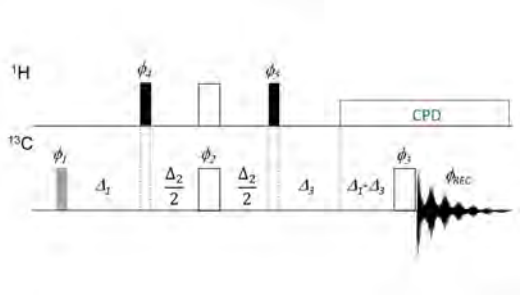
1D ^{13}C-NMR DEPT or APT experiments still belong to the most common experiments for assigning ^{13}C signals and for the elucidation of molecular structures on a routine level. The APT sequence[1] suffers from two main drawbacks, found long ago: (i) a low tolerance for wide ranges of $^1J_{CH}$ values, which in the worst-case cancels signals or produces signals with mistaken multiplicity. (ii) The frequent presence of intense artifacts in the Cq-only spectrum, especially if the range of coupling constants of the investigated molecule is large. Improved APT sequences[2] (Compensated Attached Proton Test, CAPT2 and CAPT3) have been designed by the group of McClung to improve the tolerance with respect to the wide range of $^1J_{CH}$ values. The CAPT3 sequence indeed leads to excellent tolerance over a wide range of one-bond *J* coupling constants.



In this report, we introduce a new APT sequence, the Broadband-APT (BAPT) sequence, which further improves the tolerance of the CAPT sequences to a wide range of $^1J_{CH}$ values and can provide ultra-clean Cq-only spectra, with CH_n artifact levels as low as those obtained using SEMUT, SEMUT-GL and *i*QCD sequences, known to provide the best Cq-only spectra.

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An electrochemical approach for routine radioanalytical separations

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The decommissioning of nuclear facilities, the radioactive waste management or contamination monitoring require reliable measurements of radionuclide-specific radioactivity. Such radionuclide-specific routine measurements are typically carried out with liquid scintillation counting, as well as α-, γ-, and mass-spectrometry. However, to overcome interferences arising from the different radionuclides in the sample, prior chemical separation is required. Common approaches for pre-analytical separation of radionuclides are based on ion-exchange or reverse-phase chromatography as well as precipitation/co-precipitation[1,2]. Meanwhile, methods based on electrochemistry have been scarcely used for radionuclide separations. Such approaches would provide a different selectivity, when compared to conventional methods, limit the introduction of impurities (e.g., from chromatographic resin or chemicals), and high resistance towards radiation damage. Thus, this work aims to explore the selective electrodeposition of radioisotopes of different elements as an alternative separation approach. An electroanalytical characterization of simple electrochemical systems by means of voltammetry was used to implement separation procedures based on controlled-potential electrolysis. In addition, a special electrochemical flow-through cell with a working electrode featuring a high surface area, was designed and tested for the in-flow separation of radionuclides from solutions. As a result, the approach yielded fast and efficient separations of simple mixtures of elements (i.e., their [radio]isotopes). Furthermore, the herein presented electrochemical method can be directly combined with other flow systems, such as chromatography. Therefore, the method can readily be used as a building block for more complex separation procedures and is now used for the analysis of radionuclide mixtures of increasing complexity.

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Examination of Sampling Bags for Offline Breath Analysis using Secondary Electrospray Ionization (SESI) Mass Spectrometry

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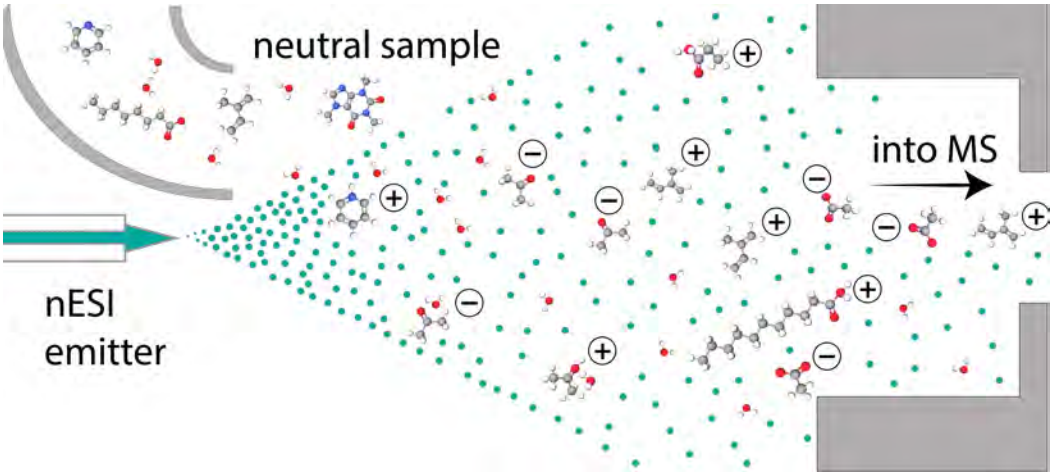
Research on exhaled breath, a fast-developing area of metabolomics, focuses on identifying disease biomarkers, tracking nutritional interventions, and analyzing the human volatilome.[1,2] It is a non-invasive, reliable and sensitive approach to diagnostics with the potential to replace invasive methods, such as blood sampling. Online analysis is the method of choice for breath analysis; however, in the clinical setting, access to a measurement facility is often limited, especially for bedridden patients. In these situations, offline methods are a viable alternative. Sampling bags are one of the most common offline sampling methods for exhaled breath.

Secondary electrospray ionization focuses on introducing an ambient gaseous sample into the ion source, where it collides with a charged nano-electrospray. The analyte molecules are subsequently ionized in the gas phase without any sample preparation or preconcentration. This approach works online and offline and is sensitive to the low-ppt range, making it a robust tool for exhaled breath analysis.[3]

In this work, we present the use of SESI coupled to a high-resolution mass spectrometer for rapid analysis of breath samples stored in bags made from various materials: Tedlar (PVF), Teflon (PTFE), Nalophan (PET), Kynar (PVDF) and EVOH (ethylene vinyl alcohol copolymer). We investigated a number of qualitative properties, such as retrievability of common breath markers, the total number of features and impurities found within each bag material.

Acknowledgements

We thank the ETH Zürich Foundation and the Departmental Mechanical Workshop for supporting this work.



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Can exhaled breath metabolomics replace rumen sampling in dairy cows?

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Previously, we characterized the intensity and daily patterns of exhaled volatile fatty acids (eVFA) using a secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS) platform. The aim of this study was to further validate the potential of the exhalomics approach to assess rumen fermentation. Four rumen-cannulated original Swiss (Braunvieh) cows were used in a switchback design with 3, 9-d periods (7-d adaptation, 2-d sampling). Cows were randomly assigned to 1 of 2 diet sequences (ABA/BAB): (A) low-starch (LS; 6.3% starch of DM), and (B) high-starch (HS; 16.2% starch of DM). Feeding was 1×/d at 0800 h. Exhalome (with GreenFeed System) and rumen samples were collected 8x to represent every 3-h of a day, and eVFA and ruminal VFA (rVFA) were analyzed using SESI-HRMS and HPLC, respectively. Data were analyzed in a mixed model with a fixed effect of the period, method, diet, and method×diet interactions, and random effect of time (repeated measures) and cow nested in sequence. Diet×method interactions were not observed. A reduced model was fitted on a method-specific subset of data to test the diet effect. The VFA molar proportions differed between HS vs. LS regardless of method: acetate was 64.1 vs. 60.1 for exhalome (P = 0.01) and 67.0 vs. 64.7 for rumen (P = 0.01), propionate 28.1 vs. 30.5 (P = 0.09) and 22.9 vs. 24.7 (P = 0.04), butyrate 7.87 vs. 9.53 (P = 0.04) and 10.1 vs. 10.7 (P = 0.11); and A:P ratio 2.49 vs. 2.14 (P = 0.05) and 3.13 vs. 2.84 (P = 0.04). For VFA daily patterns, a similar model was fitted for a diet-specific subset of data but with method×time interactions. Regardless of diet, interactions were not observed (P > 0.10). Overall, eVFA and rVFA showed similar responses to feeding and dietary treatments, indicating the potential of eVFA as a proxy to characterize rVFA molar proportions in response to dietary treatments. Future studies should further explore the potential of exhalomics in ruminant research.

Achieving Data Reduction in Space by Applying Unsupervised Machine Learning to Mass Spectrometric Data

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With the ever-increasing volume of data generated by space missions, the limitations in data downlink rates to Earth have become apparent. As the demand for scientific insights into other planetary bodies in our Solar System continues to rise, it becomes crucial to explore methods, which ensure that the data that are sent back are pertinent to the research objectives. Through optimisation of on-spacecraft data preselection, scientific returns can be maximised to ensure an efficient use of limited bandwidth resources.

In this contribution, we introduce a data reduction method based on unsupervised machine learning and clustering applied to mass spectrometric data. The input data are clustered into groups of mass spectra with similar chemical composition, corresponding to different compounds that are present in the dataset. The chemical and/or mineralogical identification of the compounds is performed on ground after the reception of representative mass spectra of each cluster. Two different yet related clustering algorithms called UMAP and densMAP are compared, as well as different levels of data pre-processing. As the method is to be applied on spacecrafts, where computing resources are limited, pre-processing should be minimal. A similar version of this clustering method was previously applied to a 1.88 Ga Gunflint sample (Ontario, Canada) to separate mass spectra recorded from the host (chert) from mass spectra containing signatures of organic matter from fossilised microbes [1].

To study the effectiveness and reliability of our machine learning procedure, data were collected using a space-prototype mass spectrometric instrument. The instrument is a compact and lightweight laser ablation and ionisation mass spectrometer (LIMS) with a femtosecond laser ion source and a reflectron-type time-of-flight (RTOF) mass analyser for the separation of ions. The instrument reaches mass resolutions exceeding 600 and lateral spatial resolutions down to 10 µm. The chemical composition of solids can be determined down to the level of mg/kg concentrations over the full mass scale [2].

The sample selected for the study is a 2.06 Ga apatite crystal obtained from an ultramafic phoscorite rock from the Phalaborwa Carbonatite Complex (Limpopo Province, South Africa). Being an accessory mineral in igneous and other rocks, apatite commonly contains a range of rare earth elements (REE), which provide valuable information for investigating physical and chemical conditions in igneous rocks and the volatile evolution of magmas [3]. By applying the clustering algorithms to the collected mass spectrometric data, three major phases corresponding to fluorapatite, forsterite and calcite were found. Additionally, several micrometre sized inclusions of baddeleyite, uranothorianite, rutile and others were detected. Given that the data were recorded using a miniature mass spectrometer designed for space flight, this analysis demonstrates the analytical capabilities of our LIMS system that could be achieved *in-situ* on other planetary bodies in our Solar System, for example on the Moon or on Mars.

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Examining the Structure-Function Relationship of Enzymes using Temperature-Controlled Nanoelectrospray Mass Spectrometry

J. A. Harrison[1], A. Pruška[1], R. Zenobi[1]*

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Tools for investigating the structural dynamics of enzymes are of great importance to researchers in academia and industry. Most solution-based techniques cannot readily provide detailed information for how the quaternary structure of a protein changes with temperature, thus limiting our understanding of their structure-function relationship. By coupling temperature-controlled nanoelectrospray ionization (TC-nESI) with mass spectrometry (MS), it is possible to measure such changes. In this work, the effects of temperature on the structure of beta-galactosidase (tetramer), jack bean urease (hexamer), and alcohol oxidase (octamer) were examined by TC-nESI. These melting data were compared with enzyme kinetics data to obtain a better understanding how of temperature-induced changes in their structure affect their function.

During the TC-nESI experiments of beta-galactosidase, the tetramer dissociated into monomer and dimer species with increasing temperature. The dimers and monomers appear at similar temperatures, suggesting that the tetramer has two dissociation pathways. Two other prominent charge state series were detected in the spectrum, which had calculated masses of 32.9 and 83.6 kDa. The sum of these masses matches the monomer mass, indicating they are fragments. This fragmentation was also found to occur slowly at 35 ℃. However, it increases rapidly with temperature. These data would explain why beta-galactosidase has been observed to lose activity over time, and rapidly with increasing temperature.

Alcohol oxidase gave rise to multiple dissociation products with increasing temperature. Monomeric species with and without FAD were detected during these experiments. The monomers without FAD appeared at lower temperatures than those with FAD, indicating that if the cofactor is not present, the complex is more readily dissociated. At higher temperatures, the alcohol oxidase complex dissociated into dimers and trimers. Previous studies on this enzyme show that this enzyme reaches its maximum activity before the dissociation of the complex, and rapidly losses activity as the complex dissociates.

When jack bean urease was examined using TC-nESI, several dissociation and aggregation products were observed. The hexamer gained high charge states, the ion mobility (IM) analysis of which indicated they are extended conformations. Monomers and trimers of different conformational states were also observed as the hexamer dissociated. Aggregates with masses corresponding to nonamers and octadecamers we also detected during these experiments. Interestingly, the temperature at which the octadecamers were at their most abundant also corresponds to the temperature at which this enzyme reaches its maximum activity. This suggests that aggregation could play a role in the activity of this enzyme.

Nuclear Forensic Investigations of High Purity Depleted Uranium Ammunition

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Sample attribution and their comparison are essential skills in a nuclear forensic investigation. Over the past two decades, the analysis of trace element impurities by sector-field inductively coupled plasma mass spectrometry (sf-ICP-MS) has become a valuable source of information. Today, lanthanoid impurities are known to be the most valuable source of information for sample attribution and comparison [1].

In this work, lanthanoid patterns, which have been extensively studied in the field of geology [2,3], are used in a nuclear forensic context to ensure reliable identification and comparison of uranium and thorium materials. Known methods lead to lanthanoid contamination and thus, to the modification of the said patterns [4]. The herein presented method addresses these shortcomings and can be used for the reliable quantification of lanthanoids. The obtained sensitivity allows lanthanoid quantification with a factor of 100 to 1000 lower, in comparison to previously established methods. This allows for a characterization of lanthanoid impurities down to the lower ppb (ng/g) to ppt (pg/g) range. Hence, the developed method is applicable to various high purity uranium materials such as nuclear fuel pellets and depleted uranium ammunition and fills an important analytical gap.

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Quantifying Total Mercury in Plankton by Cold Vapor Atomic Fluorescence Spectroscopy: Simple and Efficient Acid Digestion Procedure

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Investigating the role of plankton in mercury accumulation in aquatic food webs requires reliable procedures for mercury analysis. Established wet digestion methods exist for total mercury determination in various biological matrices, yet planktonic samples remain relatively underexplored. In response to this need, we developed a cost-effective and straightforward wet digestion method for assessing total mercury in small plankton material via cold vapor atomic fluorescence spectroscopy (CVAFS).

The digestion procedure was optimized by employing glass vessels with Teflon caps, utilizing minimal amounts of acids (either 3 mL w/w 65% HNO_3 or 3 mL 50% v/v HNO_3), maintaining a constant temperature of 85°C, and applying a continuous digestion period of 12 hours. Additionally, the protocol was tested with and without pre-ultrasound treatment in order to determine which option yields higher recoveries.

To optimize and validate the digestion procedure, we used certified reference materials IAEA-450 (unicellular alga *Scenedesmus obliquus*) and BRC-414 (plankton). The recovery efficiency of the proposed method was between 94.1 ± 7.6% and 97.2 ± 4.6% for IAEA-450 and BCR-414 (3.1 mg and 21.5 mg), respectively.

In conclusion, the method offers high recovery efficiency and precision for low-sized plankton matrices, thereby enhancing the digestion of planktonic samples for mercury analysis using CVAFS techniques.

Keywords: wet acid digestion; IAEA-450; BCR-414; plankton; microalgae; zooplankton; total mercury; CVAFS, mercury analysis

Additive Electrochemical Oxidation of Ascorbic Acid and Glucose in Enzyme Based Blood Electrochemical Meters

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Determining one's blood glucose (GL) using enzyme-based electrochemical meters is very common in a home setting to control diabetes and in treating severe situations in a hospital. Most work using a mechanism where enzymes, such as glucose oxidase or glucose dehydrogenase, present in the test strip oxidize glucose to release free electrons which are then read by electrodes in the meter **[1]**. The higher the electron current, higher the GL concentration read by the meter. Thus, this mechanism is prone to error when electrons are generated by the oxidation of interfering molecules directly at the electrode due to the potential difference between the anode and cathode. One such interfering molecule is Vitamin C (Ascorbic Acid, AA), widely used as a supplement **[2]**, consumed at very high doses in treating trauma in hospitals **[3]**, or during treatment regimes of Covid19 **[4]**. Thus, the objective is to study this proposed interference from AA on meters from five prominent manufacturers, widely available in Switzerland with differing price points. The names of the companies were kept limited to authors for confidentiality reasons. GL concentration used in this study covers the physiological range in fasted state (60-100 mg/dL), fed state (120-140 mg/dL) and diabetes. Similarly, AA covers the physiological range (1-1.5 mg/dL) as a supplement and when given intravenously (approximately 30 mg/dL).

GL reading at 60 mg/dL glucose increases with increasing AA concentration from 0.1 to 25 mg/dL. Similar observations of increasing GL readings were seen at all five GL concentrations (50, 60, 80, 100, 150 mg/dL) and using meters made by all five companies, showing a decrease in specificity due to AA, which is further substantiated when a few meters used gave GL reading with only AA solutions. Using a calibration curve of GL values with only AA solutions, the net GL reading at different AA concentrations could be explained by equation 1, which assumes an additive effect of the concentration-dependent oxidation of GL and AA. The regression line (R^2=0.98) shows good predictions from equation 1 and confirms additive effects (slope=1.06) of oxidation.

Reading on glucose meter = Value at zero AA + [AA]*slope of AA calibration curve

All meters showed a positive deviation in the glucose readings due to the direct oxidation of AA; the extent of this impact varied across the different meters. At low GL (50 mg/dL), the meter from company 1 showed the lowest percent positive error of 16% but other companies showed between a 40 and 65% error (lower specificity), with a similar pattern across the spectrum. This can be explained by the manufacturers designing a very specific patented mutated version of enzyme/co-enzyme systems and/or by advancing the electronics of the meter to increase the specificity by selectively targeting the oxidation of GL. Also, percent error, as expected from equation above, decreased with increasing glucose concentration within each company's meter; e.g. for company 5 there is an error of 65% at 50 mg/dL GL but only 19% at 150 mg/dL GL.

Conclusion: Overall, this study shows that easily oxidizable drugs in the blood will lead to a positive error, lower specificity, while determining one's glucose level and the selection which company's glucometer to use makes a significant impact on the magnitude of this error.

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Hyphenated MS-Methods as a Tool for Orthogonal Metabolite Annotation in On-Line Breath Analysis with SESI-HRMS

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On-line breath analysis with secondary electrospray ionization (SESI) coupled to high-resolution mass spectrometry (HRMS) is a powerful method for rapid and non-invasive examination of the human metabolome. Various clinical research projects have shown the effectiveness of this technique. [1] However, the identification of metabolites and biomarkers using SESI-HRMS is still limited due to the lack of a hyphenated separation method prior to the MS analysis. Comparing annotated metabolites found in exhaled breath condensate (EBC) with detected features in on-line data can merge this gap and serve as a step toward building a database of metabolites for the human exhalome. For the comparison, the breath of 16 healthy adults was measured on-line for 10 days with a SESI source (*Fossil Ion Tech*, Spain) coupled to a Q-Exactive Plus Orbitrap mass spectrometer (*Thermo Fischer Scientific*, Germany) while also simultaneously condensing part of the exhaled breath. EBC was analyzed using an Acquity UPLC system (*Waters Corporation*, USA) coupled to the same mass spectrometer, employing both reverse-phase and hydrophilic interaction columns. In addition, a data-independent MS^2-acquisition method derived from PAcIFIC [2] was utilized. GC-MS analysis was carried out using dynamic headspace vacuum transfer in-trap extraction coupled to GC-MS (DHS-VTT GC-MS) [3]. On-line and EBC MS^2 data were processed by custom workflows and EBC MS^1 data by standard workflows. An immense number of features were obtained in the LC-MS analysis, especially with the MS^2 method. Employing CANOPUS, the features were assigned to chemical classes, revealing that mostly amino acids and amines were detected in positive mode, while in negative mode predominantly amino acids and carboxylic acids were detected. The GC-MS analysis revealed mostly compounds of exogenous origin, such as additives from oral hygiene products like menthol or other fragrances, and flavoring agents. Food and beverage consumption restrictions before sampling will be needed to mitigate this issue. Comparison of the detected off-line features of both methods with the detected on-line features showed partial overlap, however, the m/z range from 150-200 needs to be better covered by the off-line methods. While these results demonstrate the potential of combined LC-MS and GC-MS analysis of breath condensate as an orthogonal annotation tool for SESI-HRMS, additional on-line fragmentation is needed for annotation with higher confidence.

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Development of an NMR Method for the Quantification of Phthalimidoperoxycaproic acid (PAP) in Tooth Whitening Products

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Introduction: Nowadays, tooth whitening is often not done by dental specialists but rather by end consumers themselves with the use of commercially available tooth whitening products.
Phthalimidoperoxycaproic acid (PAP) is a recently developed organic peroxide which has shown to be a milder alternative with less side effects compared to traditional active ingredients such as hydrogen peroxide and carbamide peroxide.[1][2] As a result, PAP based formulations have become increasingly popular in recent years as can be seen by the wide range of PAP-containing products on the market. Therefore, a reliable quantification method for PAP is important for the quality control of these rather expensive products.

Aim: The classical analytical technique for the measurement of peroxides is iodometric titration.[3] However, this method has multiple downsides. The main issue is that the method is non-selective for PAP but rather indicates the total concentration of oxidizing species in the sample. Other drawbacks are that undissolved remains of the sample can interfere with the titration and comparatively high amounts of sample (several hundred milligrams to multiple grams) are needed depending on the PAP content and the concentration of the used titrant solution.[4]
Thus, the goal of this project is to develop an NMR based analysis method — either based on direct measurement of the sample via High Resolution Magic Angle Spinning (HR-MAS) or analysis of liquid extracts — for PAP in tooth whitening products.

Results: In a first step, PAP was synthesized for use as reference and external calibration. Solution state NMR measurements on liquid extracts of tooth whitening pastes showed that PAP is detectable and quantifiable via resonance integration with high reproducibility. Furthermore, it is possible to distinguish between PAP and its decomposition product phthalimidocaproic acid. This is not only important for a reliable quantification but also sets the basis for indication of the stability of PAP in a given product. Corresponding kinetic studies were performed.
First results obtained with direct HR-MAS NMR of pastes show that the found PAP concentrations are clearly lower than the ones in liquid extracts of the same samples. This indicates that a direct measurement of PAP via the HR-MAS technique is not easily possible, as the compounds visibility seems to be at least partially suppressed by the sample matrix.

Outlook: In further experiments the reproducibility and accuracy of the quantification via liquid extracts has to be determined. Furthermore, it needs to be evaluated whether the extraction efficiency of PAP is comparable between different PAP formulations to assure a wide applicability of the method.

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