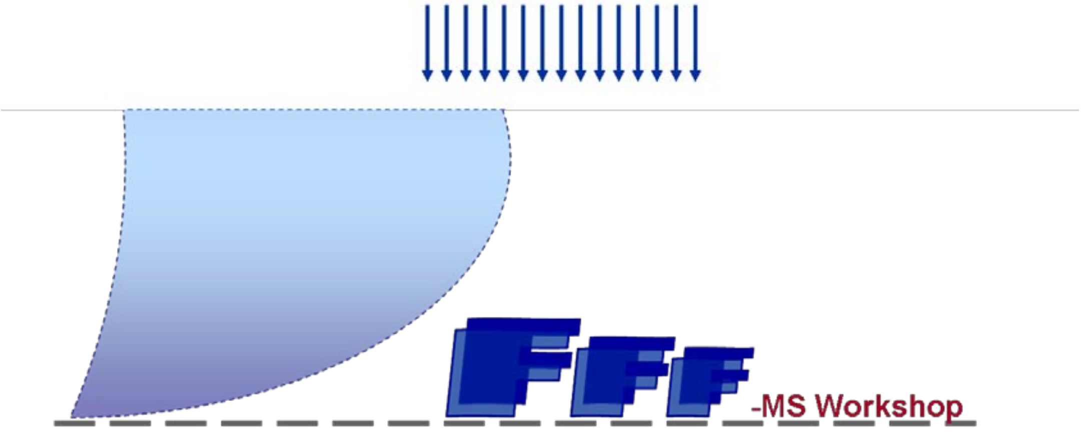
5th Workshop on Field-Flow Fractionation – Mass Spectrometry (FFF-MS)

Nanoparticle analysis in
environmental- & life-sciences
as well as
consumer products

September 28th & 29th 2017
Vienna, Austria

AGENDA & ABSTRACTS



FFF-MS workshop agenda

time	topic and content
Thursday, 28.09.2017	
08:30-08:45	**Registration & Welcome – B. Meermann, S. Wagner**
08:45-9:45	**Keynote speaker – S.A. Pergantis** Using knowledge obtained from SINGLE PARTICLE ICP-MS analysis to advance SINGLE CELL ICP-MS analysis
9:45-10:15	Coffee break
10:15-10:35	**OP1 – O. Borovinskaya** Inductively coupled plasma-time of flight-mass spectrometry as a tool for studying multi-component nanoparticles
10:35-10:55	**OP2 – J. Fuchs** Analysis of gold nanoparticles using microsecond dwell time single particle ICP-MS
10:55-11:15	**OP3 – D. Mozhayeva** Separation of Nanoparticle Mixtures with Different Sizes and Coatings by CE-SP-ICP-MS
11:15-11:35	**OP4 – A. Philippe** Potentials and challenges of hydrodynamic chromatography coupled to ICP-MS for quantifying and characterizing nanoparticles in environmental media
11:35-11:55	**OP5 - J. Bettmer** HPLC-ICP-MS for the Analysis of Nanoparticles
11:55-13:00	Lunch break
13:00-14:30	**Tutorial – F. vd Kammer** “Cruxes in nanomaterial analysis”
14:30-15:30	**Keynote speaker – R. Kaegi** Engineered nanoparticles in complex matrices: Challenges and opportunities for electron microscopy
15:30-16:00	Coffee break
16:00-16:20	**OP6 – P. Krystek** Seeing the bigger picture of nanoparticle analysis in consumer products and exposure matrices
16:20-16:40	**OP7 – M. Correia** Analysis of a mixture of nanoparticles in toothpaste by asymmetric flow field-flow fractionation coupled to inductively coupled plasma mass spectrometry
16:40-17:00	**OP8 – P. Llano** Hyphenation between AF4 with elemental mass spectrometry for characterization of nanoparticle-labelled antibodies
17:00-17:30	Final discussion
19:00	Get together in a restaurant

Friday, 29.09.2017	
8:30-9:30	Introduction and Hands on training - Method development for FFF separation and SP-ICP-MS coupling
9:30-10:00	**Coffee break**
10:00-12:00	Hands on training - Size calibration of the FFF system and characterization of poly-disperse samples
12:00-13:00	**Lunch break**
13:00-15:00	Hands on training - Technical aspects of FFF-ICP-MS
15:00-15:15	**Farewell – S. Wagner, M. Velimirovic, B. Meermann**

Abstracts - oral presentations

Keynote lecture 1 - Using knowledge obtained from SINGLE PARTICLE ICP-MS analysis to advance SINGLE CELL ICP-MS analysis

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In this presentation some recent developments for the determination of metal containing nanoparticles (NPs) using single particle (sp) inductively coupled plasma mass spectrometry (ICP-MS) will be discussed. These include new approaches for handling heavy matrix samples,[1] hyphenated techniques including hydrodynamic chromatography and field flow fractionation, alternative ways to determine sample introduction transport efficiency[2] and thus improve analysis accuracy, as well as using new sample introduction systems and their potential advantages. We will then go on to discuss how we envision these advances to help us improve the developing field of single cell (sc) ICP-MS analysis. The critical nature of experimental parameters such as cell transport efficiency, fast data acquisition and rapid data processing will be explained. As will the need for ensuring that cells maintain their integrity during their transportation into the plasma.

To better illustrate some of these points, the use of sc-ICP-MS for the determination of metal and metalloid amounts (Pb, Cd, Ni, As) in individual *Chlamydomonas reinhardtii* cells,[3] previously incubated in these metals, will be discussed. More specifically, the determined by sc-ICP-MS metal mass per cell and population will be compared with corresponding values obtained by acid digestion and total metal determination by ICP-MS. These mass balance comparisons allow for assessing the sc-ICP-MS technique in terms of its accuracy, precision and stability. The obtained metal distributions may provide us with meaningful conclusions concerning the mode of uptake of the given metals by the *Chlamydomonas reinhardtii* cells, which in turn may contribute to the advancement of our understanding of biological system function.

References:

[1] C. Toncelli, K. Mylona, I. Kalantzi, A. Tsiola, P. Pitta, M. Tsapakis, S. A. Pergantis, “Silver nanoparticles in seawater: a dynamic mass balance at part per trillion silver concentrations”, *Sci. Total Environ.* 2017, 601-602, 15-21.

[2] B. Ramkorun-Schmidt, S. A. Pergantis, D. Esteban-Fernández, N. Jakubowski, and D. Günther, “Investigation of a Combined Microdroplet Generator and Pneumatic Nebulization System for Quantitative Determination of Metal-Containing Nanoparticles Using ICPMS” *Anal. Chem.* 2015, 87 (17), 8687–8694.

[3] E. Mavrakis, N. Lydakis-Simantiris, C. Stephan, R. Magarini, S. A. Pergantis, “Investigating heavy metal (Pb, Cd, Ni and arsenic species) uptake by *Chlamydomonas reinhardtii* cells by means of Single-Cell ICP-MS” 10th Instrumental Methods of Analysis Modern Trends and Applications» (IMA 2017), Sept. 17-21, Heraklion, Greece.

Keynote lecture 2 - Engineered nanoparticles in complex matrices: Challenges and opportunities for electron microscopy

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Electron microscopy is the only method that covers the full (spatial) nano-range (1 - 100 nm), and offers the possibility to investigate structural properties (via electron diffraction) and to determine the elemental composition (via energy dispersive x-ray analyses or via electron energy loss spectroscopy) of particles at the nanoscale. For these reasons electron microscopy techniques (including scanning and transmission electron microscopy) are well suited to address challenges of environmental nanotechnology, especially regarding the detection of engineered nanomaterials in complex matrices. However, despite the overwhelming capabilities offered by electron microscopy techniques, these techniques have been originally developed to study a limited number of well selected samples in great detail and consequently, high throughput applications have not been rigorously pursued, yet. As a result, electron microscopy analyses are still very labor-intensive and rather expensive compared to other commonly used analytical techniques. Although new instrumental developments especially in combination with image analyses tools allow for a continuously increasing degree of automation, sample preparation techniques still lag behind all these new developments and ultimately preclude harvesting the full potential of electron microscopy techniques.

In this overview presentation, selected case studies on the quantification of engineered nanoparticles in complex matrices (e.g. $TiO2$ particles in landfill leachates, silver nanoparticles in wastewater treatment plants and $CeO2$ nanoparticles in sewage sludge) will be presented to demonstrate the potential but also to reveal the limitations of electron microscopy techniques in this field. Furthermore, commonly applied sample preparation methods will be discussed regarding their applicability and suitability for various matrices and new developments will be presented, dedicated to producing samples for electron microscopy analyses.

I will conclude the presentation with a brief overview of the most pressing needs required to bring electron microscopy techniques to a more reliable and quantitative level in the field of environmental nanoscience in general.

OP 1 - INDUCTIVELY COUPLED PLASMA TIME-OF-FLIGHT MASS SPECTROMETRY AS A TOOL FOR STUDYING MULTI-COMPONENT NANOPARTICLES

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The detection of very low concentrations of engineered nanoparticles in the environment presents a great challenge for exposure monitoring and risk assessment. A new technology of inductively coupled plasma time-of-flight mass spectrometry (ICP-TOFMS) has recently led to the significant breakthrough in this field, owing to its capability of simultaneous and rapid multi-element detection, essential for the analysis of individual nanoparticles and cells. The ability of single particle ICP-TOFMS to discriminate engineered nanoparticles from natural nanoparticles based on distinct element fingerprints has recently been demonstrated [1]. Using machine learning algorithms, small fractions of spiked engineered CeO_2 nanoparticles could be reliably quantified within representative soil extracts. When coupled to rapid laser ablation systems, the ICP-TOFMS can be employed for fast high-resolution imaging of trace elements and nanoparticles within biological specimens. A recent study on bioavailability and toxicity of Cu, Zn and Ni in juvenile Ceriodaphnia demonstrated detection limits in a low ppm range at the lateral resolution of 2 µm. Thanks to the simultaneous detection of all elements, concertation changes in essential metal nutrients in the tissue can be examined in correlation with nanoparticle exposure. Three-dimensional distribution can be obtained by sequentially ablating parallel 2D tissue sections and optionally employing complementary techniques to support the image reconstruction [2]. We highlight on several new application examples some important features of ICP-TOFMS for studying the behavior of engineered particles and potentially toxic metals in the environment. We present multi-element single particle ICP-TOFMS data on liver extracts from pilot whales, which were examined to reveal the natural mechanism of Hg-detoxification, and show laser ablation imaging results of liver tissues to support the sp-ICP-TOFMS findings. We also report on capabilities of the ICP-TOFMS as multi-element detector for single cell analysis.

References:

[1] A. Praetorius et al., “Single-particle multi-element fingerprinting (spMEF) using inductively coupled plasma time of flight mass spectrometry (ICP-TOFMS) to identify engineered nanoparticles against the elevated natural background in soils.” *Environmental Science:Nano* 2017.

[2] S. J. Van Malderen et al., “Three-dimensional reconstruction of the tissue-specific multi-elemental distribution within Ceriodaphnia dubia via multimodal registration using laser ablation ICP-mass spectrometry and X-ray spectroscopic techniques.” *Anal. Chem.* 2017.

OP 2 - Analysis of gold nanoparticles using microsecond dwell time single particle ICP-MS

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As the number of nanoparticle applications in technology, medicine, and consumer products increases, there is also a growing interest in the characterization of nanoparticles, engineered as well as natural occurring. Common used gold particles can be found in biosensor applications, cancer treatment, or disinfection [1]. To optimize those applications and deal with risk assessments, the particles' most important characteristics like number concentration, particle size distribution, and elemental composition need to be determined – at best by a fast and versatile method.

Most rapid techniques for particle characterization lack a specific look at the single particle itself – such as UV/vis-spectroscopy using unique properties of some nanoparticles like gold nanoparticle's light absorption through localized surface plasmon resonance. Unfortunately, these methods can only analyze the whole ensemble of particles, whereas microscopic techniques such as transmission electron microscopy or atomic force microscopy can determine properties of single particles with impressive spatial resolution. However, they call for time-consuming sample preparation and sophisticated data evaluation. A technique providing fast, robust, and reliable information on the entirety of all particles as well as the individual particle is the single particle inductively coupled plasma-mass spectrometry (spICP-MS) [2,3].

Using the spICP-MS analysis with a quadrupole-based mass spectrometer thousands of nanoparticles can be characterized right after or during synthesis in a short period of time – which allows for rapid determination of particle number concentration, size distribution and precursor consumption. Short dwell times, down to microseconds, permit to resolve the transient signal generated from a particle entering the plasma. Thus, not only allow to distinguish easily between ionic background and nanoparticle event, but may also provide additional information, if a particle event arises from a single particle, double particle or aggregate of those.

References:

[1] E.C. Dreaden, A.M. Alkilany, X. Huang, C.J. Murphy, M.A. El-Sayed, *Chem. Soc. Rev.* 2012, *41*, 2740.
[2] C. Degueldre, P.-Y. Favarger, S. Wold, *Anal. Chim. Acta* 2006, *555*, 263.
[3] I. Abad-Álvaro, E. Peña-Vázquez, E. Bolea, P. Bermejo-Barrera, J.R. Castillo, F. Laborda, *Anal. Bioanal. Chem.* 2016, *408*, 5089.

OP 3 - Separation of Nanoparticle Mixtures with Different Sizes and Coatings by CE-SP-ICP-MS

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Capillary electrophoresis (CE) is a powerful technique that enables the separation of nanoparticles (NPs) according to their charge-to-size ratio. In this presentation, it will be discussed how the coupling of CE to single particle inductively coupled mass spectrometry (SP-ICP-MS) with microsecond time resolution ensures sensitive elemental detection of single NPs and the possibility to evaluate individual profiles of detected ion clouds. Furthermore, online preconcentration techniques will be described that can be implemented to CE to decrease the detection limit for silver NPs to the submicrogram-per-liter detection range. CE-SP-ICP-MS results can be presented as two-dimensional color maps, where NPs size, size distribution, CE migration time, CE migration profile, and particle number concentration can be assessed at each chosen diagram region. [1]

Advantages and current challenges of the CE-SP-ICP-MS will be discussed. For example, CE-SP-ICP-MS with 5 µs time resolution was successfully used to separate NPs with similar sizes and different coatings present in one mixture [2]. Specifically, the separation of each component in a mixture containing 20, 40, and 60 nm citrate-coated and 40 and 60 nm polyvinylpyrrolidone (PVP)-coated NPs was feasible. This feature enables the determination of the composition of more complex mixtures of NPs and opens more opportunities in NPs production quality control and environmental samples analysis.

References:

[1] D. Mozhayeva, I. Strenge, C. Engelhard, *Anal. Chem.* 2017, 89, 7152-7159.
[2] D. Mozhayeva, C. Engelhard, *Anal. Chem.* 2017, under revision.

OP 4 - Potentials and challenges of hydrodynamic chromatography coupled to ICP-MS for quantifying and characterizing nanoparticles in environmental media

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Compared to FFF, hydrodynamic chromatography (HDC) has been relatively neglected during the intense development of analytical techniques for quantifying and characterizing nanoparticles in environmental media. This talk is an attempt to rationalize comparison between most popular separation techniques used for colloidal samples in environmental matrices. A critical comparison of separation techniques reveals that HDC and FFF are rather complementary than concurrent. Indeed, HDC is a fairly simple technique that can be easily applied to various type of particle and medium. Universal calibration renders the size determination relatively straightforward, especially compared to FFF. Furthermore, it is possible to quantify the ionic fraction, provided ions are resolved from the colloidal analytes. Furthermore, the recent studies carried out in our group and remaining challenges concerning HDC-(SP)-ICP-MS will be summarized and discussed. While the effects of the particle composition and the surface coating the effect are weak compared to other separation techniques, the diversity in terms of particle shape represent an important challenge for the sizing of unknown colloids. The use of ICP-MS in single particle mode is promising but needs further development. In addition, the potential of several more “exotic” HDC techniques will be discussed. In particular, wide bore hydrodynamic fractionation can be useful and much more straightforward than other separation techniques for case, for which it is satisfying to distinguish between a “particulate” and a “dissolved” fraction.

OP 5 - HPLC-ICP-MS for the Analysis of Nanoparticles

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Silver is widely known to exhibit bactericidal properties. For this reason, elemental silver is used for different applications including silver-coated surgical prostheses, textiles, and wound coverage. Exposure to humans may occur via different pathways, e.g., the release of silver from implant surfaces for a patient or the inhalation of silver nanoparticles at a production site. While the release of ions is assumed to reduce infections, the behavior and fate of silver in living organisms is not fully understood. To find out about possible long-term effects, the development of analytical methods for silver determination in tissue samples is required. Elemental bioimaging by means of laser ablation coupled to inductively coupled plasma-mass spectrometry (LA-ICP-MS) or microscale X-ray fluorescence spectroscopy (µXRF) is a capable strategy for the investigation of metals in biological samples. The high sensitivity and excellent detection limits of the methods permit the determination of elements in low concentration ranges. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) imaging provides complementary molecular information.

Quantitative analysis of rat tissues was performed following exposure by silver nanoparticles via the lungs. The silver concentration was determined in different organs including lung, kidney, liver and spleen using a calibration approach based on matrix-matched internal standards, which exhibit similar physicochemical properties as the samples. Furthermore, individual nanoparticles as well as their size distribution in the tissue could be determined using dedicated detection techniques in LA-ICP-MS. Finally, it was shown by these methods that macrophages strongly accumulate nanoparticles in the tissues. Spatially resolved phospholipid profiles were obtained by MALDI-MS.

OP 6 - Seeing the bigger picture of nanoparticle analysis in consumer products and exposure matrices

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Field flow fractionation (FFF) plays a cruical rol in the separation of different sized engineered nanomaterials (ENMs) prior the identification and quantification by e.g. mass spectrometry (MS). Lots of analytical questions can be studied in this way. Whenever consumer products contain ENMs or exposure matrices are of interest, a larger number of analytical techniques like particle counters and electron microscopy get relevant too. Case dedicated selections of suitable analytical techniques must be made and the method developments are often challenging issues.

This presentation focuses on analytical approaches in case studies for the identification of ENMs in consumer products and in various biological matrices after different routes of exposure.

Example:
During sanding of nano-coated surfaces, particles can be produced. These (nano)particles are identified and investigated more closely. The analytical results are relevant information in occupational exposure assessments.

OP 7 - Analysis of a mixture of nanoparticles in toothpaste by asymmetric flow field-flow fractionation coupled to inductively coupled plasma mass spectrometry

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Toothpaste is a complex mixture of chemicals including e.g. emulsifiers, surfactants, and particles such as SiO_2, Al_2O_3 (abrasives) and TiO_2 (pigment), which can be nano or micrometer sized [1]. According to the latest European Union legislation on cosmetics (2009), the use of nanomaterials in products such as toothpaste needs to be pre-approved, based on the physico-chemical properties and a safety assessment of the nanomaterial in question. Furthermore, any ingredient present in the form of nanomaterial should be properly labeled in the product [2]. In order to enforce this legislation there is an urgent need for analytical methods capable of assessing whether a product contains nanomaterials or not, i.e. reliably measuring the number-based size distribution (according to the EU definition). In this context, a method of analysis was developed in order to characterize the nano or microparticles present in commercial toothpaste, based on the simultaneous size separation of all three types of particles by asymmetric flow field-flow fractionation (AF^4).

Two sample preparation strategies were tested for analysis by AF^4: a simpler approach involving dilution of the toothpaste in aqueous solution and a more complex procedure consisting of chemical oxidation of the matrix with hydrogen peroxide. Multi angle light scattering (MALS) was used for on-line size determination of the eluting particles and inductively coupled plasma mass spectrometry (ICPMS) was exploited for selective detection of Al_2O_3, TiO_2 and SiO_2 particles. The simpler sample preparation approach, matrix dilution, was found to be unsuitable for analysis of the toothpaste particles by AF^4 resulting in unusual elution profiles and lower recoveries. The more complex procedure involving chemical oxidation was found to be crucial for separation and detection of the toothpaste particles. The obtained channel recovery based on the MALS detector was satisfactory (>80%), and this procedure was selected for further AF^4 method development. The AF^4 method was optimized for optimal recovery and particle separation in terms of carrier liquid composition and cross-flow regime. The mass-based particle size distributions of Al_2O_3 and TiO_2 particles were obtained based on the ICPMS signal and after calibration of the channel with polystyrene particle size standards. The Al_2O_3 and TiO_2 particle mass concentration was determined based on the AF^4-ICPMS fractograms using post-channel calibration with elemental standards and internal standardization.

AF^4-MALS-ICPMS was found to be a powerful method for separating and estimating the size of the particles present in the toothpaste. This talk will describe the methodological steps required for analyzing the three distinct constituent particles and highlight the challenges related to sample preparation, AF^4 separation and ICPMS detection.

Acknowledgment: The work leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement n° 604347 (NanoDefine).

References:

[1] S. Storehagen, N. Ose, S. Midha, “Dentifrices and mouthwashes ingredients and their use.” Report, Semesteroppgave 10. semester Kull V99, Seksjon for odontologisk farmakologi og farmakoterapi, 2003, Oslo, Norway.

[2] H. Rauscher, K. Rasmussen, B. Sokull-Klüttgen, “Regulatory Aspects of Nanomaterials in the EU.” *Chemie Ingenieur Technik*, 2017, 89, 224-231.

OP 8 - HYPHENATION BETWEEN AF4 WITH ELEMENTAL MASS SPECTROMETRY FOR CHARACTERIZATION OF NANOPARTICLE-LABELLED ANTIBODIES

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The development of advanced chemical analysis tools (biosensors) for the control of food security is a key objective to ensure quality in the agricultural and food sectors, promoting the progress of key productive sectors of the European Union. In this context, one of the risks of food safety in dairy production is the possible presence of natural toxins (e.g. aflatoxins). One promising approach for quality control of the food is the development of highly sensitive and selective immunoassays. Among them, one of the most promising systems make use of labelling appropiate antibodies with inorganic nanoparticles, acting as improved photoluminescent labels.

A key issue in development of the desired labelled-antibodies is the appropriate control of the bioconjugation reaction. In this regard, the hybridation of the asymmetric flow field-flow fractionation (AF4) with an inductively coupled plasma-mass spectrometry (ICP-MS) could provide invaluable capabilities and information to achieve the intended purpose.

Thus, here AF4 coupled on-line to ICP-MS is proposed as a powerful diagnostic tool for bioconjugation studies. In particular, the determination of stoichiometry in bioconjugates (between antibody (Ab) and inorganic nanoparticles (NPs)) has been monitored by such hyphenated technique. Experimental conditions have been optimized searching for an appropriate separation between the sought bioconjugates from the eventual free NPs and antibodies excesses employed during the bioconjugation reaction. Moreover, ICP-MS was selected as elemental detector to enable sensitive and reliable simultaneous quantification of the elemental constituents of the NP-Ab bioconjugates, and the free NPs and Ab.

A potential application of this technique in the enviromental and life sciences fields is the study of the bioconjugation of luminiscent inorganic nanoparticles to antibodies for the control of toxins in milk samples.

Abstracts - poster presentations

POSTER 1 - Optimization of an extraction process for the semi quantitative determination of TiO_2 nanoparticles in environmental samples using cFFF-ICP-MS/MS

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The continuously growing application of manufactured nanoparticles (NPs) raised growing concern about possible toxic and adverse effects in particularly for the environment due to the presence of such nanomaterials. Titanium dioxide (TiO_2) nanoparticles represent one of the most frequently applied nanomaterials with a production volume of at least 15 kt per year. Recent studies indicate that TiO_2 can lead to oxidative stress beside various adverse long term effects on different living organisms. To investigate the environmental impact of such particles, knowledge about their concentration as well as their size-distribution in different environmental compartments (e.g. water and sediment) is of great importance. Additionally, information about the elemental fingerprint of these particles is relevant to differentiate natural TiO_2 from manufactured nanoparticles.

A centrifugal field flow fractionation (cFFF) system has been hyphenated to multi-angle-light-scattering (MALS) and inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) and optimized for the separation and detection of TiO_2 NPs. The ICP-MS/MS has been operated in the NH_3 mass shift mode to allow a sensitive and interference free detection of Ti in the investigated NPs [1]. In addition, a process for the extraction of the nanoparticle fraction from sediment and water samples has been developed and optimized with respect to achieve a high recovery and good extraction reproducibility.

The application of the NH_3 mass shift mode, allowed the detection of ^{48}Ti as $^{48}Ti(NH_3)_6$ at m/z 150 at levels of about 10 ng L^{-1} even in the presence of several mg L^{-1} of Ca. To achieve good separation different parameters such as field strength, carrier solution composition, flow rate as well as the field strength program were optimized to provide well resolved fractograms for all kinds of TiO_2 NPs with a size detection limit of about 30 nm. To further characterize the different tested TiO_2 NPs, their individual element fingerprint has been investigated after microwave assisted digestion. Finally a new and optimized extraction process has been developed for the screening of nanoparticles in water and sediments [2].

References:

[1] J. Soto-Alvaredo, F. Dutschke, J. Bettmer, M. Montes-Bayon, D. Pröfrock, A. Prange, *Anal. At. Spectrom.* 2016, DOI: 10.1039/c6ja00079g
[2] F. Dutschke, J. Irrgeher, D. Pröfrock, *Analytical Methods* 2017, DOI: 10.1039/c7ay00635g

POSTER 2 - Natural isotopic variation of titanium in industrially used TiO2-nanoparticles assessed by (FFF) - MC ICP-MS

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Titanium is an abundant and widely distributed element in nature, usually occurring as TiO_2 in rocks, sediments or the water body. The use of manufactured TiO_2 nanoparticles (TiO_2-NPs) in products such as pigments, sunscreens and cosmetics is constantly increasing, as is their release into the environment. In consequence, TiO_2-NPs have been recognized as potential emerging environmental contaminants. While the importance of the titanium isotopic composition in cosmological studies for the investigation of isotopic anomalies and cosmogenic effects as well as extra-terrestrial samples has been recognized since the 1960s, the natural terrestrial isotopic abundance variation is still hardly explored.

The potential of using the isotopic composition of Ti in TiO_2 nanoparticles to differentiate their sources of origin was investigated by analysis of different commercially available TiO_2 nanomaterials using solution-based MC ICP-MS (Nu Plasma II, Nu Instruments, Wrexham, UK). Calibration was performed by external intra-elemental correction for instrumental isotopic fractionation (standard sample bracketing) using NIST SRM 3162a (titanium standard solution, NIST, Gaithersburg, US) as 0-anchor for relative isotope ratio measurements. The influence of matrix components on all Ti isotope abundance ratios (expressed as $\delta^{50}Ti/^{47}Ti$, $\delta^{49}Ti/^{47}Ti$, $\delta^{48}Ti/^{47}Ti$, $\delta^{46}Ti/^{47}Ti$) present as impurities (e.g. Ca, Cr, Mg, Al, Zr, Fe, Si, V) in the nanomaterials was investigated in various spiking experiments. Relative within-run repeatabilities of measured $^{49}Ti/^{47}Ti$ ratios were in the range of 0.0002 %. The relative differences between the natural Ti isotopic compositions of the investigated standards and nanoproducts showed a variation in $\delta^{49}Ti/^{47}Ti$ of up to 1.4 ‰.

Initial results of TiO_2-NP analysis followed by data reduction applying finite mixture models and multiple linear regression show promising results for mixtures of different NP products. Therefore, the presented data shows for the first time the potential to differentiate between some of the individual TiO_2 nanoparticle products based on their individual isotopic composition, also for coupling FFF to MC ICP-MS.

POSTER 3 - Characterization of silver nanoparticles in spray disinfectant products using asymmetrical flow field flow fractionation in combination with UV/vis detection

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Given the potential for inhalation exposure of silver nanoparticles, from spray disinfectant products, we analyzed 22 commercial products which advertised the use of silver or colloidal silver as the active ingredient. Primary characterization included determination of total silver content by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), fractionation between ionic and particulate silver (ICP-OES after ultrafiltration) and measurement of particle hydrodynamic diameter using Nanoparticle Tracking Analysis (NTA) and Dynamic Light Scattering (DLS).

A number of the AgNP products showed the presence of nano-sized silver, while other products contained only ionic silver or no measurable silver at all. A high degree of variability between claimed and measured values for total silver was observed. Only 8 of the products showed total silver concentrations within 20% of their nominally reported values. In addition, significant variations in the relative percentages of particulate vs. soluble silver was also measured in many of these products reporting to be colloidal. Although the total silver concentrations were variable among products ranging from 1.6 mg/L to 466 mg/L, silver containing nanoparticles were identified in all except one of the product suspensions.

Because AgNP products showed differing degrees of polydispersity and analysis especially for the size distributions measured by DLS and NTA techniques have been shown to be biased toward larger particle sizes, we employed asymmetrical flow field flow fractionation (AF4) in combination with UV/Vis detection for advance characterization of the hydrodynamic properties of the AgNP products.

POSTER 4 - Using Relative Heteroaggregation Attachment Efficiency Factors to Assess the Role of Surface Coatings on the Fate of Engineered Nanomaterials

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Investigations into the processes affecting the environmental fate of engineered nanomaterials (ENMs) indicate that their colloidal stability, i.e., their ability to resist aggregation, can strongly influence their transport within environmental systems [1]. Focusing on aquatic environments, the concentration of natural colloidal particles is expected to be orders of magnitude greater than the concentration of ENMs [2]. This suggests that the potential for ENMs to heteroaggregate upon destabilization would be an important physical process impacting their environmental fate. In contrast to this, it has been postulated that under typical environmental conditions, the characteristic time for heteroaggregation will be relatively short in comparison to other phenomena affecting the environmental transport of ENMs, such as large-scale sediment transport processes [3]. This hypothesis is based upon the assumption that heteroaggregation attachment efficiency factors (α_{hetero}) are essentially unity and thus heteroaggregation will be rapid and complete. However, there have been few studies examining ENM heteroaggregation within actual environmental systems on which to base this hypothesis. Furthermore, it neglects the large body of research indicating that various, interrelated factors can increase the colloidal stability of ENMs [4].

Often, examinations into the colloidal stability of ENMs utilize synthetic mediums [5]. These synthetic mediums, designed to mimic environmentally-relevant conditions, enable mechanistic investigations into the factors influencing ENM behavior. This approach is highly useful when trying to identify the specific processes or factors that may be affecting the colloidal stability of an ENM. However, a growing body of research suggests that under real-world environmental conditions, many processes can simultaneously modify the properties of ENMs. These numerous biological and chemical processes can in turn alter the behavior of the ENMs. When utilizing a simplified, synthetic medium, it is currently impossible to recreate the entire breadth of these diverse processes [2] Therefore, there is a critical need to test and utilize procedures that allow investigations into how ENMs may behave within natural environmental systems.

Applying a modification of the experimental procedure outlined by Barton *et al* (2014), relative heteroaggregation attachment efficiency factors ($\alpha_{\mathrm{hetero,rel.}}$) were determined for 12-15 nm gold-core nanoparticles (AuNPs) dispersed within samples from a local freshwater river (Willamette River, Oregon, USA). Furthermore, the influence of different engineered surface coatings (2 kDa PEG, 3 kDa PEG-COOH, 3 kDa PEG-Amine, citrate, and bPEI) on the aggregation behavior of the AuNPs was investigated. Preliminary results suggest that within a complex environmental matrix, such as the freshwater environment investigated in this research, variations in the aggregation behavior of the ENMs are observable and dependent upon the initial properties of the surface coating. The overall significance of these differences in aggregation behavior on the overall environmental fate of the ENMs is still being assessed. Future research will expand to include a range of aquatic sources and utilize 'aged' ENMs that more closely mimic the transformations ENMs may experience prior to their release to the environment. This will enable more realistic investigations into how variations in the properties of the natural aquatic medium and the ENM surface coatings can affect the aggregation behavior and thus the environmental fate of ENMs.

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POSTER 5 - Characterization of nano-TiO_2 in commercial sunscreens via Inverse Supercritical Fluid Extraction and Miniaturized Asymmetrical Flow Field-Flow Fractionation hyphenated with UV, MALS and ICP-MS

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Ambiguous media coverage about the pros and cons of “nano-enhanced” consumer products in recent years has significantly propelled the discussion about their safety. With this ongoing discussion, regulatory authorities such as the European Commission launched several regulations dealing with the declaration of products, which contain nanomaterial ingredients. One is the “European Regulation EC No 12232009 of 30 November 2009 on cosmetic products, in which it is stated that “all ingredients present in the form of nanomaterials shall be clearly indicated in the list of ingredients. The names of such ingredients shall be followed by the word ‘nano’ in brackets” [1]. However, until now, there is still a clear lack of available analytical methodologies, which can provide a straightforward and reliable testing procedure for such products.

We herein present a novel approach to reliably assess the nanoparticulate content of commercially available sunscreens. This approach encompasses a mild and environmentally friendly removal of water and lipophilic sunscreen ingredients via inverse supercritical carbon dioxide extraction (scCO2) followed by the determination of the size distribution as well as the elemental composition of the nanoparticulate content via miniaturized Asymmetrical Flow Field-Flow Fractionation hyphenated with UV, Multi-Angle Light Scattering and Inductively-coupled Plasma Mass Spectrometry (mAF4-UV-MALS-ICP-MS) [2].

This setup enables a straightforward and clear distinction of “non-nano sunscreens” from “nano sunscreens” with high confidence under environmentally friendly conditions and has the potential to be the testing procedure of choice, when it comes to the verification of the “nano-labelling” of commercially available cosmetic products.

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POSTER 6 - Asymmetrical Flow Field-Flow Fractionation hyphenated with ICP-MS for Trace Level Analysis of Engineered Silver Nanomaterials in River Water

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Asymmetrical Flow Field-Flow Fractionation (AF4) is a powerful tool for the separation of nanomaterials according to their hydrodynamic size. However, significant dilution within the separation channel usually limits its application towards trace level analysis. These limitations can be overcome by taking advantage of two unique features, which this separation technique can offer:

1) Slot outlet

Here, the channel outlet flow is split into two separate flows. Since the sample is usually located close to the semipermeable accumulation wall in the lower 5-10% of the channel, the upper, sample-free, void volume can be removed thereby reducing sample dilution.

2) High volume injection (HVI)

In AF4, a focusing or relaxation step is necessary prior to elution, where sample components arrange in different heights of the separation channel according to their different diffusion coefficients counteracting with an external force field. This is realized by a second flow, the so called cross flow, which acts perpendicular to the channel flow and pushes the sample components towards the acumulation wall. During sample introduction, a focus flow, which counteracts the sample injection flow, hereby enables virtually unlimited sample introduction and thus sample enrichment and cleaning directly on the separation channel.

A third mean to prepare AF4 for trace analysis is the coupling with a high-sensitivity detector:

3) Hyphenation with ICP-MS

ICP-MS is particularly powerful, when it comes to the chemical identification and quantification of metallic and metal oxidic sample components such as e.g. silver nanomaterials (AgNM). Hence, its hyphenation with AF4 (AF4-ICP-MS) enables the collection of data on elemental composition over the size distributions even at trace level concentrations.

In this presentation, we demonstrate the applicability of AF4-ICP-MS for the quantification of AgNM spiked in Rhine water at trace level concentrations. Using an injection volume of 8 mL together with a 60% removal of the upper channel flow, we were able to identify and quantify AgNM down to a LOQ of 14 ng/L.

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POSTER 7 - Investigating Heterogeneous Nanoplastics in Environmental Samples Using Asymmetric Flow Field Flow Fractionation Coupled with Online Detectors

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The impact of nanoplastics (NPs) on the environment is a growing concern. The proportion of plastic marine litter composed of nano-sized particles (NP; defined here as material having at least one size measurement between 1 and 100nm) is uncertain. Although studies report cases of plastic nanoparticles affecting algal photosynthesis and growth, altering fish metabolism and behaviour and causing malformation in zooplankton, it is difficult to quantify exposure concentrations and the environmental risk is poorly known [1, 2]. Hence there is a drive to develop a deeper understanding of the fate and behaviour of NPs.

Several groups have investigated the applicability of the novel analytical separation technique Asymmetric Flow Field Flow Fractionation (AF4) to separate heterogeneous NP. AF4 has been coupled with various detectors including ICP-MS to build a reliable tool for the identification of engineered nanoparticles (ENPs) [3, 4, 5]. However, there is a need to adapt, optimise and critically assess these methods for nanoplastics studies in more problematic sample types, such as natural waters.

Here we investigate the applicability of AF4 to separate nonuniform NPs sampled in a wide range of aquatic environments. The poster will present the initial development and optimisation of a NP separation method that uses NIST-certified polystyrene nanospheres of various sizes in environmentally relevant matrices as well as the results of preliminary separation work using natural water samples. This project aims to provide valuable information on the emission of NP to the environment, its environmental fate, exposure to aquatic life and potential toxic effects.

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POSTER 8 - Considerations in the characterization and analysis of engineered nanomaterials in exposure matrices

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As nanotechnology is still an emerging field with an increasing variety of engineered nanomaterials (ENMs), products and applications, there is a great relevance in assessing health and environmental hazards. This presentation focuses on analytical approaches for the identification of ENMs in products and in various biological matrices after different routes of exposure.

A project dedicated selection of exposure matrices and analytical techniques must be made. The analytical challenges of a few cases will be presented in details.

- For exposed tissues, the matrix dissolution prior to measurement by asymmetric flow field flow fraction hyphenated to inductively coupled plasma mass spectrometry (AF4-ICPMS) is challenging because the ENMs must stay stable regarding to particle size and composition. A new enzymatic approach is explored in comparison to the classical dissolution approaches in speciation analysis.
- Next to *in vivo* studies, *in vitro* cell exposure testing has been put forward as a faster approach to screen ENMs on their toxic potential but also to study the possible cellular uptake which depends on the characteristics of the ENMs. The analysis delivers new insights into the understanding of ENMs in mainly complex media. Additional aspects, like e.g. the composition of the cell culture medium that affects aggregation of ENMs and this in turn may affect exposure levels, are of great relevance.
- Other examples by imaging techniques likes scanning electron microscopy (SEM) will be illustrated. In this case, we focussed on nanoparticles which are produced during sanding of nano-coated surfaces.

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POSTER 9 - Combination of Electrical and Flow Field-Flow Fractionation to Measure Electrophoretic Mobility of Nanoparticles and Proteins

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A new FFF method is presented which combines asymmetrical Flow-FFF (AF4) and Electrical FFF (ElFFF) in one channel (EAF4) [1]. The new method allows to measure electrophoretic mobility of eluting species. An AF4 channel is modified by adding two electrodes, one at the upper wall, the other beneath the frit of the accumulation wall. The measurement consists of a series of FFF separation experiments in which the electrical field strength is varied by applying different electrical currents, starting from 0 to typically 10 to 20 mA. A shift of retention time as a function of current or field strength is observed and from a plot of the electrical field strength versus drift velocity the electrophoretic mobility is obtained. The method is straightforward for homogeneous size populations where the shift in retention time of the peak maximum is taken for the mobility calculation. It can be used for populations with a broad size distribution if the size is measured by light scattering (either MALS or DLS). In this case, the mobility as a function of size can be calculated across the distribution. The method has been validated by comparing mobility values measured by EAF4 and PALS for well-known particle standards [1]. In this contribution, we present results of the analysis of samples with a broad size distribution showing that the zeta potential is changing with size. Possible application of the method for the analysis of complex samples which contain species of different charge and polarity are discussed.

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POSTER 10 - Nanoparticles Separation Using Capillary Electrophoresis

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Capillary electrophoresis (CE) is an analytical technique that separates analytes based on their electrophoretic mobility with the use of an applied voltage.

Nanoparticles separation using CE exhibits good separation and analysis efficiency, especially for nanoparticles smaller than 30 nm. The coupling of CE with inductively coupled plasma mass spectrometry (ICP-MS) provides the possibility to detect nanoparticles in the concentration range of nanograms per litre and enables the simultaneous detection of nanoparticles and its ionic counter parts [1, 2].

Our objective is to develop a fast and reliable separation method for differently modified nanoparticles using capillary electrophoresis.

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