Final program

TraceSpec 2007

11th Workshop on Progress in Analytical Methodologies for Trace Metal Speciation

September 4-7, 2007
Münster, Germany

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Organization of TraceSpec 2007

Organizers

WWU - The University of Münster
Institute of Inorganic and Analytical Chemistry

EVISA
The European Virtual Institute for Speciation Analysis

IAEAC
The International Association of Environmental Analytical Chemistry

International Scientific Committee

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M. Sperling (Co-Chair), EVISA, Germany
M. Filella (Co-Chair), University of Geneva, Switzerland
J. A. C. Broekaert, University of Hamburg, Germany
O. F. X. Donard, University of Pau, France
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T. Hoffmann, University of Mainz, Germany
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Sponsors

Our particular thanks go to the sponsors of the conference, who have helped to keep registration affordable.

Thermo Scientific

PerkinElmer LAS (Germany) GmbH

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AHF analysentechnik AG

Welcome to TraceSpec 2007 and welcome to Münster!

On behalf of the Scientific Committee of TraceSpec 2007 and the supporting organizations, the European Virtual Institute for Speciation Analysis (EVISA), the University of Münster and the International Association of Environmental Analytical Chemistry (IAEAC), it is an honor and pleasure to warmly welcome you to the City of Münster. The workshop will provide an international forum to share knowledge and to exchange ideas with colleagues from all over the world.

Speciation analysis is a highly interdisciplinary topic, and this will be reflected by five plenary lectures, 38 contributed lectures and over 80 posters. Technologically-oriented presentations will cover the latest developments with respect to analytical separations coupled to selective spectroscopic and mass spectrometric detectors as well as electrochemical developments, just to mention a few. Applications include the fields of food analysis, environmental analysis, biomedicine and many others. The technical program will be complemented by an exhibition of instrumentation by the most important manufacturers in the field.

A conference, which is focused on a specific topic like speciation analysis needs to promote interaction between the participants. Therefore, we have planned ample time for discussions after the oral presentations as well as in the poster and exhibition halls. To allow intense discussions close to the technical program, lunch will be provided on-site, and the social program with a welcome reception, a Westphalian evening and a barbecue will motivate to refresh existing and to establish new connections.

As young scientists will be the future of our discipline, we particularly try to stimulate their participation by low conference fees for students, to recognize their important contributions by several poster prizes including the well-renowned Roland W. Frei award and to incorporate several PhD students into the lecture program.

Financiation of such a conference is an important issue. Therefore, our particular thanks go to the sponsors and the exhibitors of the conference, who helped to keep registration affordable.

Let us all look forward to an event in the beautiful City of Münster, which will stay lifelong in your memory!

Uwe Karst
Chairman

Michael Sperling
Director of EVISA

Dieter Klockow
President of the IAEAC

General Information

Conference Venue
University of Münster
- Castle -
Schlossplatz 2
48149 Münster, Germany

How to Reach the Conference Venue?
The castle is situated close to Münster's historic city center. A large parking lot (parking toll has to be paid) is located close to the conference venue. The castle can be reached by the following bus lines:
Line **5** (direction "Nienberge Hannaschweg"): get off at bus stop "Überwasserstraße"
Line **5** (direction "Hiltrup Bahnhof"): get off at bus stop "Neutor"
Line **6** (direction "Kinderhaus Brüningheide"): get off at bus stop "Überwasserstraße"
Line **6** (direction "Hiltrup Bahnhof"): get off at bus stop "Neutor"
Lines **11, 12, 13, 22** (both directions): get off at bus stop "Landgericht"
The recommended bus stop is "Landgericht", which is located just a 3 minutes walk south of the castle. Bus stop "Neutor" is located in a 10 minutes walking distance northeast, and bus stop "Überwasserstraße" is located in a 5 minutes walking distance east of the conference venue. Bus tickets (a single ticket is € 2.00, a four-trips ticket is € 7.10) can be bought at the ticket machine or at the driver (ticket machines are only available at selected bus stops; ticket machine display can be switched to English). Please make sure to have cash money (preferably coins) with you. Payment by credit card or EC (Maestro) card is not possible. Four-trips tickets have to be validated in the bus.

Conference Office / Registration Desk
The conference office / registration desk is located in the entrance hall of the university castle. Opening hours are:

Tuesday,	September 4, 2007	13:30 - 21:00
Wednesday,	September 5, 2007	8:00 - 18:00
Thursday,	September 6, 2007	8:00 - 18:00
Friday,	September 7, 2007	8:00 - 14:00

Cloakroom
A surveilled cloakroom is located in the basement of the castle (follow the signs downstairs). Wardrobe and luggage can be deposited there during the opening hours of the conference office.

Name Badges
TraceSpec 2007 participants are kindly requested to wear their name badges throughout the conference. In case that you should have lost your badge, a new one is available at the registration desk.

Smoking
Within the university castle - as a for all university buildings - smoking is prohibited. It is kindly requested to smoke outside the building.

Short Course
The short course “Speciation analysis - the practical way” (by O. X. F. Donard, University of Pau, France) will be given in the Senators lounge on the first floor (please follow the signs in the conference building) on Tuesday, September 4, 2007 from 14:00 - 17:00.

Lectures
The single-stream lecture program will be held in the castle’s auditorium on the first floor. Please follow the signs in the conference building. Morning speakers are kindly requested to provide their presentation on USB by the end of the day before their lecture will be given. Afternoon speakers are kindly requested to provide their presentation on USB by the beginning of the lunch break. TraceSpec 2007 staff taking care of your presentation will be available in the auditorium.

Poster Sessions
Poster sessions take place in lecture hall 6 on the first floor (please follow the signs in the conference building). Poster group I shall be mounted on Tuesday evening, but at the latest until Wednesday, September 5, 2007 at 8:30. Poster group I authors are kindly requested to be present at their posters on Wednesday, September 5, 2007 from 15:40 to 16:40. Poster group I should be removed by Wednesday, September 5, 2007 at 18:15 and may be stored at the registration desk. Poster group II shall be mounted until Thursday, September 6, 2007 at 8:30. Poster group II authors are kindly requested to be present at their posters on Thursday, September 6, 2007 from 15:40 - 16:40. Poster group II should be removed on Friday, September 7, 2007 before the end of the conference.

Exhibition
TraceSpec 2007 features an exhibition of instrumentation and equipment, which provides an excellent opportunity to present the latest developments in the field of speciation analysis and to bring together customers and manufacturers. Exhibitor booths of the following companies are located in the entrance hall: Agilent Technologies, AHF analysentechnik AG, Gerstel, MLS - Milestone, PerkinElmer, PS Analytical Ltd., Shimadzu, Thermo Scientific. Exhibition is open on Wednesday and Thursday from 9:00 to 17:00, and on Friday from 9:00 to 13:00.

Coffee and Lunch Breaks
Coffee, tea and refreshments will be served next to the exhibition in the entrance hall throughout the conference. During lunch breaks, there will be a buffet with a delicious selection of warm and cold food, which is also served in the exhibition hall. Coffee and lunch breaks are included in the registration fee.

Internet
For the convenience of the participants, LAN/WLAN will be provided free of charge during the workshop both in the exhibition hall as well as in the Senators Lounge on the first floor (follow the signs in the conference building). The installation will require that participants use their own notebooks on which they will need administrator rights for Windows XP. Other versions of the Windows system (e.g. Windows Vista) will not be supported. Login, password and a short description of the installation procedure will be provided by the workshop office on request.

Bus Connections to the City Center and to Münster Central Station
The fastest way to go to the city center is a 10 minutes walk from the castle. However, the bus stop next to the conference venue is "Landgericht" (when you leave the castle at the main entrance, turn right and walk approx. 250 m straight ahead while passing the parking lot. Cross the street at the traffic light. All buses departing from that side of the street go to the city center and to Münster central station). For bus ticket purchase, please refer to "How to reach the conference venue?". Information about the bus schedule is available at the conference office.

Bus Connections from Münster Central Station to Münster-Osnabrück International Airport (FMO)
Buses regularly ride from Münster central station to FMO airport. Buses to FMO take approx. 45 min. Bus line S50 departs from platform B3 always at 21 min past the hour (scheduled transfer time: 35 min). Bus line R51 departs from platform B3 always at 9 min to the hour (scheduled transfer time: 46 min). A ticket to FMO airport is € 5.40.

Train Connections from Münster Central Station to Düsseldorf International Airport (DUS)
A ride to Düsseldorf International Airport by train takes approximately 90 to 110 min. (For most train connections from Münster to Düsseldorf airport, you have to change the train one time). Further information can be found on the Deutsche Bahn (German Rail) website: http://reiseauskunft.bahn.de/bin/query.exe/en

Cash Dispensers
Cash dispensers next to the conference venue are:

Sparkasse Münsterland Ost
Münzstraße 1 - 3
48143 Münster (approx. 10 min walking distance from the conference venue)

Postbank
Domplatz 6 - 7
48143 Münster (approx. 20 min walking distance from the conference venue)

Citibank
Rothenburg 35
48143 Münster (approx. 20 min walking distance from the conference venue)

Post office
The post office next to the conference venue is:

Deutsche Post
Domplatz 6 - 7
48143 Münster (approx. 20 min walking distance from the conference venue)

Taxi
To call a taxi, dial: +49 251 60011 ("Taxi Münster"). In case that you should need support, TraceSpec 2007 staff at the conference office will be happy to assist you.

TraceSpec 2007: Program Overview

Tuesday, September 4	Wednesday, September 5	Thursday, September 6	Friday, September 7
	8:00 Registration desk	*8:00 Registration desk*	*8:00 Registration desk*
	***8:15* Opening of the workshop**		
	8:40 **Plenary lecture PL1**	*8:30* **Plenary lecture PL3**	*8:30* **Plenary lecture PL5**
	9:20- 10:20 Contributed presentations: Environmental speciation analysis	*9:10-10:10* Contributed presentations: Tools for speciation analysis	*9:10-10:10* Contributed presentations: Speciation analysis in proteomics, metallomics and metabolomics
	10:20-11:00 Coffee break in the exhibition area	*10:10-10:50* Coffee break in the exhibition area	*10:10-10:50* Coffee break in the exhibition area
	11:00-12:40 Contributed presentations: Environmental speciation analysis	*10:50-12:30* Contributed presentations: Tools for speciation analysis	*10:50-12:30* Contributed presentations: Speciation analysis in proteomics, metallomics and metabolomics
13:30-21:00 *Entrance hall, Registration desk: On-site registration*	*12:40-14:00* Lunch break in the exhibition area	*12:30-14:00* Lunch break in the exhibition area	***12:30*** **Announcement of Poster awards**
14:00-17:00 **Senators lounge: Short course SC1**	*14:00* **Plenary lecture PL2**	*14:00* **Plenary lecture PL4**	***12:50*** **Closing ceremony**
Registration desk: On-site registration	*14:40-15:40* Contributed presentations: Speciation analysis for the food and health sector	*14:40-15:40* Contributed presentations: In-situ speciation analysis and environmental speciation monitoring	
16:00-16:30 Coffee break	*15:40-16:40* Coffee break / **Poster session I**	*15:40-16:40* Coffee break / **Poster session II**	
Registration desk: On-site registration	*16:40-18:00* Contributed presentations: Speciation analysis for the food and health sector	*16:40-18:00* Contributed presentations: In-situ speciation analysis and environmental speciation monitoring	
18:00-21:00 **Entrance hall: Get-together mixer**	*18:15* **Meeting at the main entrance: „Westphalian Evening“**	*18:15* **Meeting at the main entrance: Guided city tour “Münster jovel”**	

Wednesday, September 5, 2007

Morning session 1: Environmental speciation analysis
Chairman: Uwe Karst (University of Münster)

8:15		Opening of the workshop: Uwe Karst, Dieter Klockow, Michael Sperling
8:40	**PL 1**	Plenary lecture: Olivier F. X Donard (University of Pau, France): Environmental chemistry of elements - elemental speciation at last!
9:20	**OP 1.1**	Petra Krystek (former at RIVM, The Netherlands): Monitoring of chromium species and eleven selected metals in emission and immission of airborne environment
9:40	**OP 1.2**	Markus Lenz (Wageningen University, The Netherlands): Bioremediation of selenium containing waters: Importance of selenium speciation analysis
10:00	**OP 1.3**	María Jiménez Moreno (University of Castilla-La Mancha, Spain): A general strategy for monomethylmercury analysis: from low to highly polluted sediments
10:20		*Coffee break and exhibition*
11:00	**OP 1.4**	Stephan Hann (University of Natural Resources and Applied Life Sciences Vienna, Austria): High priority contaminants of the aquatic environment: Speciation analysis for detection and the evaluation of removal strategies
11:20	**OP 1.5**	Patrick Thomas (Institute Pasteur of Lille, France): Environmental speciation analysis: A practical viewpoint and some applications in a contract laboratory
11:40	**OP 1.6**	Hans A. van der Sloot (Energy Research Centre of the Netherlands, ECN, The Netherlands): Chemical speciation fingerprints by geochemical modeling after the characterization of the leaching behavior of various metals
12:00	**OP 1.7**	Ruth E. Wolf (United States Geological Survey, Denver, USA): Simultaneous speciation of As, Se, and Cr: Feasibility for real samples
12:20	**OP 1.8**	Shona McSheehy (Thermo Fisher Scientific Bremen, Germany): The Determination of PBDEs by GC-ICP-Q-MS
12:40		*Lunch break and exhibition*

Afternoon session 2: Speciation analysis for the food and health sector
Chairman: Thorsten Hoffmann (University of Mainz, Germany)

14:00 **PL 2** Plenary lecture: Erik H. Larsen (National Food Institute, Technical University of Denmark, Søborg, Denmark): The role of trace element speciation in life-science research

14:40 **OP 2.1** Emma Warburton (LGC Ltd., Middlesex, UK): Microwave-assisted enzymatic hydrolysis: Evaluation of its capabilities for Se-speciation

15:00 **OP 2.2** Claudia Swart (Federal Institute for Materials Research and Testing (BAM), Berlin, Germany): Stabilization of As-species in water using different SPE materials

15:20 **OP 2.3** Patcharin Jankong (Mahidol University Bangkok, Thailand): Arsenic accumulation and speciation in freshwater fish living in arsenic-contaminated waters

15:40 *Coffee break and poster session I*

16:40 **OP 2.4** Erwin Rosenberg (Technical University of Vienna, Austria): LC/ESI-MS study of the structure of germanium sesquioxide (Ge-132) in aqueous solution

17:00 **OP 2.5** Stefan Trümpler (University of Münster, Germany): Gas chromatography coupled to microwave-induced plasma emission detection (GC-MIP-PED) for the speciation analysis of mercury

17:20 **OP 2.6** Petra Cuderman (Jožef Stefan Institute, Ljubljana, Slovenia): Determination of Se species in Se enriched and drought exposed potatoes by HPLC-ICP-MS and HPLC-UV-HG-AFS

17:40 **OP 2.7** Ray B. Voegborlo (Kwame Nkrumah University of Science and Technology, Kumasi, Ghana): Determination of methylmercury in fish from the marine and freshwater environments in Ghana using gas liquid chromatography with electron capture detection technique

18:00 *End of session 2*

18:15 *Meeting at the main entrance for the „Westphalian Evening“*

Thursday, September 6, 2007

Morning session 3: Tools for speciation analysis
Chairman: Olivier F. X. Donard (University of Pau, France)

8:30 **PL 3** Plenary lecture: Klaus G. Heumann (University of Mainz, Germany): Hyphenated isotope dilution ICP-MS as an accurate tool for speciation analysis

9:10 **OP 3.1** Pablo Rodríguez-González (LCABIE, Pau, France): Comparison of different numerical approaches for multiple spiking species-specific isotope dilution analysis exemplified by the determination of butyltin species in sediments

9:30 **OP 3.2** Jan Kösters (Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany): Beyond GC/ICP-MS: New tools for elemental speciation of volatiles

9:50 **OP 3.3** Mirko Peitzsch (Institute for Geoscience, University of Mainz, Germany): Cryotrapping-cryofocussing-GC-ICP-MS (CT-CFGC-ICP-MS) analysis of microbial volatilized selenium sorbed on soil materials

10:10 *Coffee break and exhibition*

10:50 **OP 3.4** Heiko Hayen (ISAS, Dortmund, Germany): Investigation of non-covalent metalphytosiderophore species by electrospray-high resolution mass spectrometry

11:10 **OP 3.5** Ralf Kautenburger (Saarland University, Germany): CE-ICP-MS as speciation technique to analyze the complexation behavior of lanthanides with humic acid

11:30 **OP 3.6** Bernhard Kuczewski (Technical University of Graz, Austria): CE-DAD-ICP-MS as tool for investigation in the complexation of metals and for metal speciation

11:50 **OP 3.7** Christian Dietz (Complutense University of Madrid, Spain): Comparison of miniaturized sample treatment systems for As speciation in human hair and nails

12:10 **OP 3.8** Montserrat Filella (University of Geneva, Switzerland): Trace element speciation modeling applied to environmental systems: The deceiving reality and some new perspectives

12:30 *Lunch break and exhibition*

Afternoon session 4:
In-situ speciation analysis and environmental speciation monitoring
Chairperson: Montserrat Filella (University of Geneva, Switzerland)

14:00	**PL 4**	Plenary lecture: Martial Taillefert (Georgia Institute of Technology, Atlanta, USA): Combining in-situ measurements with speciation techniques to characterize biogeochemical processes in sediments
14:40	**OP 4.1**	Günter Weber (ISAS, Dortmund, Germany): Direct monitoring of metabolic Fe-species equilibria in plants by CV and MS
15:00	**OP 4.2**	Corinne Parat (LCABIE, Pau, France): Determination of trace metal speciation parameters by using screen-printed sensors in stripping chronopotentiometry
15:20	**OP 4.3**	Josep Galceran (Universitat de Lleida, Spain): AGNES: Determining free Zn concentration in seawater
15:40		*Coffee break and poster session II*
16:40	**OP 4.4**	Maria Pesavento (Università di Pavia, Italy): Speciation investigation of trace metal ions using complexing resins as sensors for the free metal ion concentration in real samples
17:00	**OP 4.5**	Ana Maria Almeida Mota (Centro de Química Estrutural, Lisboa, Portugal): Copper speciation in different types of natural waters
17:20	**OP 4.6**	Latif Elçi (University of Pamukkale, Turkey): Inorganic arsenic speciation in various water samples with GF-AAS using coprecipitation
17:40	**OP 4.7**	Kerstin Leopold (Technical University of Munich, Germany): Flow-injection system for ultra trace analysis of mercury species by AFS
18:00		*End of session 4*
18:15		*Meeting at the main entrance for the guided city tour "Münster jovel"*

Friday, September 7, 2007

Morning session 5: Speciation analysis in proteomics, metallomics and metabolomics
Chairman: Erwin Rosenberg (Vienna University of Technology, Austria)

8:30	**PL 5**	Plenary lecture: Alfredo Sanz-Medel (University of Oviedo, Spain): MS-based proteomics turns quantitative: The great future of ICP-MS in that undertaking
9:10	**OP 5.1**	Gerhard A. Wiesmüller (Umweltprobenbank des Bundes, Münster, Germany): Trace element species and its impact on human health
9:30	**OP 5.2**	Susanne Bomke (University of Münster, Germany): Metallocenes as labels in bioanalysis
9:50	**OP 5.3**	Stefan Pieper (Humboldt University, Berlin, Germany): MeCAT: Applications for absolute quantification of proteins and peptides using HPLC/ICP MS
10:10		*Coffee break and exhibition*
10:50	**OP 5.4**	David Point (NIST, USA): Use of dual spike speciated isotope dilution to investigate the differences of reactivity between freeze dried and cryogenic biological standard reference materials for mercury speciation analysis
11:10	**OP 5.5**	Frank Hasenäcker (University Duisburg-Essen, Germany): Online coupling of 2D-HPLC and CN-PAGE for native separation of metalloproteins with subsequent detection by LA-ICP-MS
11:30	**OP 5.6**	Aleksandra Polatajko (ISAS, Dortmund, Germany): Analytical assay development to study metalbinding proteins in plants
11:50	**OP 5.7**	Maria Rosario Fernández de la Campa (Department of Physical and Analytical Chemistry, Oviedo, Spain): HPLC-ICPMS and ESI-MS to study the possible synergic effect of cadmium and selenium in induced metallothionein
12:10	**OP 5.8**	Gunda Köllensperger (University of Natural Ressources and Applied Life Sciences, Vienna, Austria): Measuring metallodrug-peptide/protein interaction by speciation analysis
12:30		**Poster awards**
12:50		*Closing ceremony*

Poster session I:
Environmental studies (1) / Studies in the field of biosciences (4)

Environmental studies (1)

PO 1.1 **Evaluation of two strains of Portuguese marine cyanobacteria in natural seawater: Biological response and chemical speciation of the culture medium**
Adriano Fachini, M. R. F. Martins, V. M. O. Vasconcelos and M. T. S. D. Vasconcelos (Interdisciplinary Centre for Marine and Environmental Research, CIIMAR, Porto, Portugal)

PO 1.2 **Mending of contaminated seawaters using marine cyanobacteria. Evaluation of the trace metals removal rates and chemical speciation of the medium**
Joana P. N. Ribeiro and Adriano Fachini (Universidade do Porto, Portugal)

PO 1.3 **Development and validation of a method for the quantification of tributyltin at subnanogram per liter concentrations**
Christian Piechotta, Thomas Sommerfeld, Tin Win and Irene Nehls (Federal Institute for Materials Research and Testing, Berlin, Germany)

PO 1.4 **Chemical fractionation in investigations of heavy metals mobility in industrial waste polluted soils**
Aleksandra Bielicka, Katarzyna Swierk and Irena Bojanowska (University of Gdansk, Poland)

PO 1.5 **Distribution and speciation of metals in a polluted site of Brasilicata (Southern Italy)**
Claudia Belviso, Francesco Cavalcante, Saverio Fiore, Spartaco Di Gennaro, Luca Medici, Achille Palma and Pietro P. Ragone (Istituto di Metodologie per l'Analisi Ambientale – IMAA, Tito Scalo, Italy)

PO 1.6 **Mercury pollution surveys in Riga (Latvia)**
Zanda Gavare, Egils Bogans, Atis Skudra and Anda Svagere (University of Latvia, Riga, Latvia)

PO 1.7 **Selective ultratrace determination of uranium isotopes in the environment**
Sebastian Raeder, P. Schuhmann, K. Wendt, B. Bushaw, N. Trautmann and J. V. Katz (Johannes Gutenberg-Universität, Mainz, Germany)

PO 1.8 **Multielement speciation of mercury and tin in inland surface waters using GC-ICP-HR-MS**
Shona McSheehy, Torsten Lindemann, J. Wills, T. Oki and Meike Hamester (ThermoFisher Scientific, Bremen, Germany)

PO 1.9 **Iodine speciation in the marine aerosol by inductively coupled plasma isotope dilution mass spectrometry**
S. Lai, J. Heilmann, W. Brüchert, A. Helfrich, J. Bettmer and T. Hoffmann (Johannes Gutenberg–University, Mainz, Germany)

PO 1.10 Determination of atmospheric iodine species using a diffusion denuder and GC-MS technique
Ru-Jin Huang, Sen-Chao Lai, Marc-Christopher Reinnig, Jörg Warnke and Thorsten Hoffmann (University of Mainz, Germany)

PO 1.11 On-line speciation and determination of Cr(III) and Cr(VI) in drinking and waste water samples by reversed-phase high performance liquid chromatography coupled with atomic absorption spectrometry
Deniz Yurtsever Sarica, A. Rehber Turker and Esra Erol (The Scientific and Technological Research Council of Turkey -TUBITAK, Ankara, Turkey)

PO 1.12 Mercury speciation in contaminated sediments using headspace trap gas chromatography and atomic fluorescence spectrometry
Baghdad Ouddane, Nevenka Mikac, Jean Claude Fischer and Gabriel Billon (University of Sciences and Technology of Lille, France)

PO 1.13 Chromium(VI) speciation through the environment
Katrine Aspmo, Hilde T. Uggerud, Marit Vadset and Andreas Woldegiorgis (Norwegian Institute for Air Research, Kjeller, Norway)

PO 1.14 Determination of Cr(VI), selected heavy metals, and elemental carbon in PM10 from a roadside sampling side in Vienna City
Harald Hagendorfer, Sebastian Koeppel and Andrea Hanus-Illnar (Karl-Franzens-University Graz, Graz, Austria)

PO 1.15 Speciation in environmental samples: A small review
M. Carmo Freitas, Zdenka Šlejkovec, Hubert Th. Wolterbeek and Adriano M. G. Pacheco (Technological and Nuclear Institute, Sacavém, Portugal)

PO 1.16 The use of isotope dilution gas chromatography - mass spectrometry for the determination of butyltin species in marine environmental samples
Tadeja Milivojevič Nemanič, Janez Ščančar and Radmila Milačič (Jožef Stefan Institute, Ljubljana, Slovenia)

PO 1.17 Mercury species stability in crude oil studied by species specific isotope dilution GC-ICP-MS
Lars Lambertsson, Charles J. Lord, Erik Björn and Wolfgang Frech (Umeå University, Sweden)

PO 1.18 Studies of transport and collection characteristics of gaseous mercury species in natural gases using amalgamation and isotope dilution analysis
Tom Larsson, Wolfgang Frech and Erik Björn (Umeå University, Sweden)

PO 1.19 Separation and determination of mercury sulfide and mercury bound to the organic matter in river sediments
Qichao Wang, Na Zheng and Shaoqing Zhang (Chinese Academy of Sciences, Changchun, China)

PO 1.20 Determination of mercury species in Portuguese salt marshes using capillary GC - atomic fluorescence spectrometry
Warren T. Corns, Peter B. Stockwell, Derek W. Bryce, M. V. Válega, E. Pereira and M. Pardal (presented by Bin Chen, PS Analytical Ltd.)

PO 1.21 Use of different simple methods for the estimation of radium concentration in a variety of environmental samples
Zornitza Tosheva, S. Chalupnik, N. Dimova and A. Kies (University of Luxembourg, Luxembourg)

PO 1.22 Chromium speciation in environmental samples using Dowex M 4195 chelating resin
Kadriye Ozlem Saygi, Mustafa Tuzen, Mustafa Soylak and Latif Elci (Gaziosmanpasa University, Tokat, Turkey)

PO 1.23 Speciation and quantification of mercury in contaminated soils of the rural area of Descoberto – Minas Gerais, Brazil
Cláudia Carvalhinho Windmöller, Walter Alves Durão Júnior, Helean Eugênia Leonhardt Palmieri (UFMG, Belo Horizonte, Brazil)

PO 1.24 The determination of hexavalent chromium in industrial and environmental samples
Fabienne Séby, Jaime Pacheco and Olivier Donard (Ultra Traces Analyses Aquitaine, Pau, France)

PO 1.25 Determination of phytoremediation capability of selected plant species (atriplex nitens and descurainia sophia) for lead contamination
Dilek Demirezen Yilmaz, Cem Vural and Ebru Vural (Erciyes University, Kayseri, Turkey)

PO 1.26 Water pollution determination of Cd, Pb, Cu and Zn in water by GF-AAS and F-AAS
Cristina Ţoca, Elisabeta Bianu and Daniela Nica (Institute of Diagnosis and Animal Health, Bucharest, Romania)

PO 1.27 Determination of chromium in wastewater, drinking water and soil contaminated by tanneries, Sialkot (Pakistan)
Uzaira Rafique, Ayesha Ashraf, Sumreen Iqbal and Irum Asif (Fatima Jinnah Women University, Rawalpindi, Pakistan)

PO 1.28 Trace metal analysis in root crops and its fields of Islamabad (Pakistan)
Uzaira Rafique, Hira Kaukab, Sumreen Iqbal and Irum Asif (Fatima Jinnah Women University, Rawalpidi, Pakistan)

Studies in the field of biosciences (4)

PO 4.1 **Speciation data from voltammetric methods: Ni in xylem sap – a case study**
Sheila Alves, Margarida Maria Correia dos Santos and Maria de Lurdes Simões Gonçalves (Instituto Superior Técnico, Lisboa, Portugal)

PO 4.2 **Optimizing conditions for labelling proteins with 2-(4-isothiocyanatobenzyl)- 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid**
Larissa Wäntig and Norbert Jakubowski (Institute for Analytical Sciences, Dortmund, Germany)

PO 4.3 **High resolution ICP-MS trace element analysis of protein fractions obtained from size exclusion high pressure chromatography of human cerebrospinal fluid**
Kristin Gellein, Lars Evje, Trond Peter Flaten and Tore Syversen (Norwegian University of Science and Technology, Trondheim, Norway)

PO 4.4 **Analysis of cisplatin adducts to oligonucleotides of enzymatically digested DNA using HPLC-ESI-Ion trap-MS**
Shereen Mowaka and Michael Linscheid (Humboldt University Berlin, Germany)

PO 4.5 **Interactions of peptides and proteins with arsenic species and metal ions: Investigations by means of electrospray ionization mass spectrometry (ESIMS)**
Anne-Christine Schmidt and Matthias Otto
(Technical University Bergakademie Freiberg, Germany)

PO 4.6 **Exhalation of trimethylbismuth after oral application of a bismuth salt – evidence for biomethylation of metals in the human body**
Jens Boertz, Frank Mosel, Margareta Sulkowski, Albert W. Rettenmeier and Alfred V. Hirner (University Duisburg-Essen, Germany)

PO 4.7 **Detection of Ni-species and Co-species in single living plant cells with high lateral resolution**
Yvonne Scheller and Heinz Duschner (Johannes Gutenberg-University Mainz, Germany)

PO 4.8 **A new enzyme-assay for PLA2 activity in jellyfish venom based on phosphorus detection using HPLC-CC-ICP-MS**
Anja Zimmermann, Heike Helmholz, Daniel Pröfrock and Andreas Prange (GKSS Research Centre Geesthacht, Germany)

PO 4.9 **Speciation of selenium in animal tissues using high performance liquid chromatography with on-line detection by inductively coupled plasma mass spectrometry**
Eliza Kurek, Anna Ruszczyńska, Marcin Wojciechowski and Ewa Bulska (Warsaw University, Poland)

PO 4.10 **Speciation analysis of selenoproteins from human serum by affinity chromatography hyphenated to inductively coupled plasma-mass spectrometry**
Petru Jitaru, Marco Prete, Giulio Cozzi, Warren Cairns, Paolo Cescon and Carlo Barbante (Institute for the Dynamics of Environmental Processes (CNR), Venice, Italy)

Poster session II: Studies in food science (2) / Tools for speciation analysis (3)

Studies in food science (2)

PO 2.1 **Mercury speciation in shell fishes from Korea**
Cheolho Yoon, Hyeon Yoon and Yun-Cheol Na (Seoul Center, Korea Basic Science Institute, Seoul, Korea)

PO 2.2 **Assessment of the heavy metals in the food from Romania, 2005 - 2006**
Carmen Hura and B. A. Hura (Institute of Public Health, Iasi, Romania)

PO 2.3 **Methylmercury determination in fish samples by GC-ICP-MS and species-specific isotope dilution**
Stefan Trümpler, Reinhard Kruse, Wolfgang Frech and Wolfgang Buscher (University of Münster, Germany)

PO 2.4 **Extraction behavior and speciation of arsenic in Wakame**
Regina Kirsch and Jürgen Mattusch (Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany)

PO 2.5 **Determination of methylmercury in fish muscle by GC-AFS**
Rostislav Červenka, Josef Komárek and Veronika Czibulková (Masaryk University, Brno, Czech Republic)

PO 2.6 **Arsenic and its species in total diet supplied from Slovenian Forces**
Vekoslava Stibilj, Zdenka Šlejkovec, Zdenka Trkov and Barbara Orešnik (Jožef Stefan Institute, Ljubljana, Slovenia)

PO 2.7 **Determination of butyltin species in seafood samples by ultrasonic probe extraction and isotope dilution analysis by GC-MS**
M. Sánchez, Manuela Hidalgo, D. Sánchez-Rodas and J. L. Gómez-Ariza (University of Girona, Girona, Spain)

Tools for speciation analysis (3)

PO 3.1 A new peroxidase POX1B, biochemical characterization, suitable biosensor for hydrogen peroxide detection in biological samples
El Ichi Sarra, Abdelghani Adnane, Hadji Imene, Helali Salwa, Limam Farid and Marzouki M. Nejib (INSAT, Tunis, Tunisia)

PO 3.2 Use of a microwave plasma torch coupled to electrochemical hydride generation for the optical emission spectrometric determination of As
Martin Amberger and José A. C. Broekaert (University of Hamburg, Germany)

PO 3.3 Enzymatic probe sonication treatment in cadmium plants determination
Ana Maria Mota, T. Armas, F. Vilhena, J. L. Capelo and M. L. Goncçalves (Instituto Superior Técnico, Lisboa, Portugal)

PO 3.4 Role of dynamic metal speciation and membrane permeability in the metal flux at permeation liquid membranes
Zeshi Zhang and Jacques Buffle (University of Geneva, Switzerland)

PO 3.5 Influence of inorganic complexes on the transport of trace metals through PLM
Stéphane Bayen, Peggy Gunkel-Grillon and Jacques Buffle (Université de Genève, Switzerland)

PO 3.6 Vanadium speciation by chromatographic separation of V(IV) and V(V) in acidic solution followed by ICP-OES determination
Paul Coetzee and Mingsong Hu (University of Johannesburg, South Africa)

PO 3.7 Isoelectric focussing of small metal-species
Günther Weber, Helma Geltenpoth and Heiko Hayen (Institute for Analytical Sciences, Dortmund, Germany)

PO 3.8 Speciation of mercury and organomercury compounds by high performance liquid chromatography/electrospray ionization mass spectrometry
Yun-Cheol Na, Hyeon Yoon, Cheolho Yoon and Jung-Ju Seo (Korea Basic Science Institute, Sungbuk-Gu, Korea)

PO 3.9 Determination of methyl mercury of biological samples by SPME-GC-ICP-MS
Burkhard Knopf, Mirko Peitzsch, Daniel Kremer and Helmut König (Johannes Gutenberg University, Mainz, Germany)

PO 3.10 Different extraction procedures comparison for the evaluation of antimony mobility in soils from an abandoned Sb-mining area
Giovanna Armiento, Cinzia Crovato, Elisa Nardi, Renata Pacifico and Elisa Petrini (ENEA, Roma, Italy)

PO 3.11 Application of mercury determination by Zeeman atomic absorption spectroscopy in forensic analysis
Egils Bogans, Zanda Gavare, Natalia Zorina and Gatis Bebris (University of Latvia, Riga, Latvia)

PO 3.12 Selective ionophore-based optical sensor for metal ion measurement in aqueous environments
Li Li and Fiona Regan (Dublin City University, Dublin, Ireland)

PO 3.13 Minimization of systematic errors in ultra trace analysis of mercury species with FI-CV-AFS
Lena Harwardt, Kerstin Leopold and Michael Schuster (Technische Universität München, Germany)

PO 3.14 Head-space solid-phase microextraction of butyl- and phenyltin compounds in human urine after derivatisation with sodium tetraethylborate and subsequent determination by capillary gas chromatography with microwave-induced plasma atomic emission and mass spectrometric detectors
George A. Zachariadis and Erwin Rosenberg (Aristotle University, Thessaloniki, Greece)

PO 3.15 Hyphenation of capillary LC with ICP-MS and on-line micro fraction collection for MALDI-TOF analysis as complementary tools for protein analysis
Daniel Pröfrock and Andreas Prange (GKSS Research Centre Geesthacht, Germany)

PO 3.16 Multiplexed probing of cytochromes P450 using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)
Arunchalam Venkatachalam, C. U. Koehler, I. Feldmann, J. Messerschmidt, A. Manz, P. H. Roos and N. Jakubowski (ISAS, Dortmund, Germany)

PO 3.17 Identification of arsenobetaine degradation products by means of HPLC – parallel ICPMS- and ESIMS-detection
Jürgen Mattusch, Daniela Möller, Maria P. Elizalde-Gonzalez and Rainer Wennrich, (UFZ - Centre for Environmental Research, Leipzig, Germany)

PO 3.18 Evaluation of enzymatic-assisted extraction protocols for the analysis of total arsenic and arsenic species from individual leaves of terrestrial plants by means of IC/ICPMS and ICPAES
K. Kutschera, A. C. Schmidt, Jürgen Mattusch and Matthias Otto (Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany)

PO 3.19 Speciation of alkylphenols after labeling with ferrocene
Nina Kolbe and Jan T. Andersson (University of Münster, Germany)

PO 3.20 Application of nuclear instrumentation methods for the characterization of diffusion membranes
Zornitza Tosheva and D. Klein (University of Luxembourg)

PO 3.21 Determination of the surface coverage of DNA conjugated gold nano-particles by simultaneous determination of gold and phosphorus
Andy Scheffer, Thorben Pfeifer, B. Yamilet Mejia-Radillo, Ernesto Carillo-Nava, Hans-Jürgen Hinz and Wolfgang Buscher (University of Münster, Germany)

PO 3.22 Automated multiple determination of Hg-species in marine biota by GC-CVAFS after TMAH digestion and solvent stripping
Reinhard Kruse (Lower Saxony Federal State Office for Consumer Protection and Food Safety, Cuxhaven, Germany)

PO 3.23 SC-FAST: Fully automated low pressure systems for performing trace elemental speciation by ICP-MS
Dan Wiederin, Paul Watson and Patrick Sullivan (Elemental Scientific, Omaha, USA)

PO 3.24 Investigation of diffusive gradients in thin films technique applicability for mercury speciation measurements
Pavel Diviš, Lukáš Brulík and Hana Dočekalová (Brno University of Technology, Brno, Czech Republic)

PO 3.25 Potential for the speciation of Al in human serum using convective interaction media (CIM) fast monolithic chromatography with ICP-MS and cap nano LC ESI-MS detection
Simona Murko, Radmila Milačič, Janez Ščančar and Bogdan Kralj (Jožef Stefan Institute, Ljubljana, Slovenia)

PO 3.26 Comparison of external calibration and isotope dilution in the determination of tributyltin (TBT) in seafood by GC-ICP-MS
Stig Valdersnes, Amund Maage, Berit Solli and Kåre Julshamn (National Institute of Nutrition and Seafood Research, Bergen, Norway)

PO 3.27 Focused-microwave assisted extractions for speciation of Hg, Sn, Cr and As from environmental samples
Jaime Pacheco-Arjona, Pablo Rodríguez-González, Sylvain Bouchet, David Amoroux, Fabienne Seby, David Braclay and Olivier X. Donard (Chimie Analytique Bioinorganique et Environnement , Pau, France)

PO 3.28 Spray chamber optimization for the coupling of CE to ICP-MS
Björn Meermann, Andy Scheffer, Marc Bartel, Martin Vogel and Uwe Karst (University of Münster, Germany)

PO 3.29 Single cell quantification of platinum in Chinese hamster ovary cells treated with cis-platinum (cis-diamminechloroplatinum(II)) using LA-ICP-MS: A new tool for the comparative analysis of single CHO-9 cells by LA-ICP-MS and optical microscopy
Sebastian D. Müller, Alfred V. Hirner and Wolfgang Goedecke (University Duisburg-Essen, Essen, Germany)

PO 3.30 Determination of germanium sesquioxide (Ge-132) by gas chromatography with microwave-induced plasma atomic emission after derivatisation
Evaggelia Tzannetou, Nikolaos S. Thomaidis and Erwin Rosenberg
(Vienna University of Technology, Vienna, Austria)

PO 3.31 New approach of transmission electron microscopy coupled with EDXS for heavy metal speciation in environmental/biological materials
V. P. Singh, Saurabh Verma, Vinod Kumar Tarar and Sashi N. Kumar
(Indian Council of Medical Research, New Delhi, India)

PO 3.32 Analysis of arsenic compounds by CE/ESI-ToF-MS and CE/ICP-MS
Marc Bartel, Björn Meermann, Andy Scheffer, Martin Vogel and Uwe Karst
(University of Münster, Münster, Germany)

PO 3.33 Analysis of Gadolinium-based MRI Contrasting Agents by CE/ESI-ToF-MS and HILIC/ESI-MS
Jens Künnemeyer, Faruk Tokmak and Uwe Karst
(University of Münster, Germany)

Poster Awards

Roland W. Frei Poster Award

It is a main endeavor of IAEAC to support and stimulate young scientists' research activities. Therefore, IAEAC has established the Roland W. Frei Poster Award, which regularly goes to young researchers presenting outstanding results on their posters. At TraceSpec 2007, the best poster out of poster sessions I and II will be awarded the Roland A. Frei Poster Award, which is endowed with € 700,-. An electronic version of the awardee's poster will later be published on the IAEAC website (www.iaeac.ch).

Poster Awards for Poster Sessions I and II
Within both poster sessions, two posters each will be awarded poster prizes that have been sponsored by the following companies:

1st prize in poster session I (endowed with € 500,-):	Thermo Scientific
2nd prize in poster session I (endowed with € 300,-):	PerkinElmer LAS (Germany) GmbH
1st prize in poster session II (endowed with € 500,-):	AHF analysentechnik AG
2nd prize in poster session II (endowed with € 300,-):	PS Analytical Ltd.

Poster Award Session
The poster award session is to be held on Friday, September 7, 2007 at 12:30 in the auditorium hall.

Conference History

Since 1983, IAEAC together with local organizers has held a series of workshops related to trace metal speciation. To date, ten meetings of this type have been organized throughout Europe and in Canada. Starting in 2000, with the eighth meeting of the series, analytical chemistry was made the focal point of the workshop as indicated by the change of its title into "Progress in Analytical Methodologies for Trace Metal Speciation". TraceSpec 2007 is also following this concept and presents the most recent research results in the field of speciation analysis as well as future strategies suited to face upcoming challenges.

Chronology of Trace Metal Speciation Workshops:

1983	1st Workshop:	Carcinogenic and/or Mutagenic Metal Compounds (Environmental Chemistry, Analytics, Biological Effects), Geneva/Switzerland
1986	2nd Workshop:	Carcinogenic and/or Mutagenic Metal Compounds, Villars-sur-Ollon/Switzerland
1988	3rd Workshop:	Toxic Metal Compounds (Interrelation between Chemistry and Biology), Follonica/Italy
1991	4th Workshop:	Toxic Metal Compounds (Interrelation between Chemistry and Biology), Les Diablerets/Switzerland
1994	5th Workshop:	Metals and Genetics, Toronto/Canada
1995	6th Workshop:	Metal Compounds in Environment and Life, Jülich/Germany
1997	7th Workshop:	Metal Compounds in Environment and Life, Modena/Italy
2000	8th Workshop:	Progress in Analytical Methodologies in Trace Metal Speciation, Lisbon/Portugal (part of EUROANALYSIS XI)
2002	9th Workshop:	Progress in Analytical Methodologies in Trace Metal Speciation, Dortmund/Germany (part of EUROANALYSIS XII)
2005	10th Workshop:	Progress in Analytical Methodologies in Trace Metal Speciation, Luxembourg/Luxembourg
2007	11th Workshop:	Progress in Analytical Methodologies for Trace Metal Speciation: TraceSpec 2007, Münster/Germany

Social Program

Get-Together Mixer on Tuesday, September 4, 2007

All TraceSpec 2007 participants are kindly invited to join exhibitors and organizers for a get-together mixer from 18:00 to 21:00 in the entrance hall on Tuesday. Drinks, refreshments and snacks will be served during this reception.

Westphalian Evening on Wednesday, September 5, 2007

For those participants having registered for this event, the Westphalian evening is an excellent opportunity to enjoy Münster's hospitality combined with a typical Westphalian atmosphere. Starting at 18:15 near the conference building, buses will take you to the planetarium, which is situated next to the lake "Aasee" and Münster's zoo. Discover "The Secrets of the Southern Hemisphere" during a 45 minutes presentation based on impressive photographies taken by ESO's (European Organization for Astronomical Research in the Southern Hemisphere) observatory in desert Atacama (Chile). Afterwards, there will be a short walk to the so-called "Mühlenhof", an assembly of historic Westphalian buildings, where the conference dinner will take place subsequent to a welcome "Korn", a typical Westphalian liquor. The "Mühlenhof" is an outdoor museum opened in 1961. It assembles a variety of historic buildings, e.g., a windmill, a forge, small farm houses, and stables. The conference dinner will be in the so-called "Gräftenhof". Originally, the "Gräftenhof" - a typical arrangement of Westphalian farm houses - had been situated 7 km away before it was being reconstructed on its current place from 1973 - 1976.

Guided Tour "Münster Jovel" to the City Center and TraceSpec 2007 Barbecue on Thursday, September 6, 2007

All TraceSpec conferees are kindly invited to join the guided tour "Münster jovel" to the city center starting at 18:15 on Thursday, September 6, 2007. During this tour, you will learn many interesting - and sometimes astonishing - facts about Münster's history, the city's curiosities and famous buildings, and, of course, about Münster's population. Surely, afterwards you will agree that Münster is a "jovel" (a word typically used in Münster for "super, nice, interesting") city! After your return to the conference venue at around 19:45, we start the TraceSpec barbecue in front of the building – another opportunity to meet and discuss in a pleasant atmosphere.

About

IAEAC

The International Association of Environmental Analytical Chemistry (IAEAC) is a world-wide operating organization that aims to promote and maintain scientific excellence in analytical science as applied to different environmentally relevant research areas. Not only the traditional compartments soil, water, and air are included in its activities, but likewise fields such as industrial processes, human health, or quality of food. In these efforts, the IAEAC strongly collaborates with relevant disciplines other than chemistry.

In order to achieve its goals, the IAEAC provides opportunities for scientists to exchange “cutting-edge” research results significant for the environment and to become acquainted with the most advanced analytical technologies. This is successfully practiced through regularly occurring events such as symposia, workshops, and short courses, now over more than 35 years.

More information about the IAEAC can be found on: ***www.iaeac.ch***

EVISA

The European Virtual Institute for Speciation Analysis (EVISA) is a service provider in the fields of speciation analysis. EVISA's web portal is the primary source for all those seeking information about chemical species with respect to analysis, biological activity (toxicity, nutritional value, metabolism), legislation (laws, rules, standards) and research in related fields. The Virtual Institute is meant to be the web-portal for all those, seeking information about chemical species and their determination. In order to fulfill its mission, EVISA will provide different services, meant to

- promote the dissemination of information and knowledge toward industry and formulate new requirements as a feed-back from industrial demands,
- help industry to install speciation related solutions fit-for-purpose to solve real world issues,
- enhance the discussion between analytical scientists,
- improve the interdisciplinary cooperation between scientists from different fields, such as toxicology, biology, medicine, nutritional science, earth sciences, environmental sciences,
- improve the quality and traceability of speciation analysis by performing different quality related actions,
- improve the education of analytical scientists for speciation analysis and quality assurance related to speciation,
- enhance the visibility of speciation related science toward policy and rule makers by adding the European dimension,
- inform policy and rule makers about the state-of-the-art of the current academic knowledge to facilitate the establishment of most effective legislation considering the characteristics of element species,
- act as a promoter of new research strategies emerging from the realization of speciation
  demands in industrial, food, health, and environmental issues.

More information about EVISA can be found on: ***www.speciation.net***

About

University of Münster
Founded in 1780 as "Vicecancellarius Universitatis", the University of Münster looks back on plenty of history. In the first half of the 19th century, the Prussian government transformed the university into an academy. In 1902, the Prussian king and German emperor Wilhelm II. adjudicated the university title back to the academy. Therefore, since 1907, the university's official name is "Westfälische Wilhelms-Universität Münster (WWU Münster)". In 1908, only a few years prior to World War I, the first women were allowed to study at the WWU Münster. In 1925, the medical faculty has been established. Severely damaged during World War II, the university had to be closed in the winter semester of 1944/1945. Reopened already in November 1945, the university was rapidly growing and a large number of new disciplines was established. Today, the university's 40,000 students make it the third biggest university in Germany. One important argument for students' decision to study at the WWU is the enormous teaching on offer: 130 courses form a broad portfolio of options. The University of Münster confers 700 to 800 PhDs a year, making it one of the five most important institutions in Germany for producing junior research staff. The WWU's research profile is marked by a considerable number of research focal points of proven excellence – in the humanities (including the theological faculties), law, business administration, mathematics, medicine and of course the natural sciences.

For further information, please visit: ***www.uni-muenster.de/en/***

Münster – Facts and Figures
Founded more than 1200 years ago by the Frisian missionary Liudger, the city has a long history as the seat of the bishops of Münster. In 1648, Münster became a city of peace and the birthplace of the modern Netherlands when the Treaty of Westphalia was signed here as well as in the city of Osnabrück, thus ending the disastrous Thirty Years' War. After the Napoleonic era, the city became the capital of the Province of Westphalia. Severely damaged in World War II, Münster was rapidly rebuilt in the 1950s, and today, the combination of old street network and modern buildings attracts a large number of tourists form all over the world. In 2004, Münster has been awarded the title of "World's Most Liveable City" (LivCom Award 2004).

With 280,000 inhabitants in 2006, Münster is an important economic and scientific center in the northwestern part of Germany. It can easily be reached by car, train and plane. Münster/Osnabrück International Airport (FMO) is situated just a short 40 minutes drive from the city center. The city hosts the University of Münster and the University of Applied Sciences, both together currently educating some 50,000 students in more than 180 disciplines. Furthermore, Münster is a home of established research institutes that are part of the Max-Planck Society or the Leibniz Association, respectively.

Paper Submissions for a Special Issue of
International Journal of Environmental Analytical Chemistry

TraceSpec 2007 organizers would like to invite all speakers and poster presenters to submit their work for publication in a special issue of the International Journal of Environmental Analytical Chemistry.

The scope of the journal comprises such subjects as original research on all aspects of analytical work related to environmental problems such as analysis of organic, inorganic and radioactive pollutants in air and water; determination of harmful substances, including their metabolic breakdown products; and analytical methods for metabolic breakdown patterns or other chemical degradation patterns in the environment and in biological samples. The journal also covers the development of new analytical methods or improvement of existing ones useful for the control and investigation of pollutants or trace amounts of naturally occurring active chemicals in the environment, or methods that help to gain more insight into our environment. Development, modification and automation of instruments and techniques with potential in environmental sciences are also part of the journal.

The journal editor is J. Albaigés (Barcelona/Spain).

Manuscript submission deadline for the special issue is October 31, 2007.

All manuscripts have to be submitted electronically via: http://mc.manuscriptcentral.com/geac. “Instructions to authors” are also available on this website. Please use “TraceSpec 2007” as a key-word in order to indicate that your manuscript is for publication in this special issue.

Please note that all manuscripts submitted to International Journal of Environmental Analytical Chemistry will undergo full peer review, based on initial editor screening and anonymized refereeing by at least two expert referees.

PL 1

Environmental chemistry of elements: Elemental speciation at last

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It is well recognized that metals and metalloids occur in the environment under a large array of chemical species. These species have different chemical properties which control their fate, transformation, reactivity and translocation between the different compartments of the environment. It also controls their toxicity. At present, our understanding of trace elements only relies on total concentrations and hence and transfer between different compartments of the environment is mostly realized on global budget assessment.

Sustainable development requires a precise and accurate assessment of the information related to elements and their species. We cannot any more by-path this information for sound environmental management, safe control of food or effective process control in industry. If we compare the knowledge that we now have on the fate of organic contaminants we are much more advanced in understanding the chemical properties, reactivity and transfer in the environment. Global models based on their chemical species allow anticipating the global fate and impacts of the organic species in the environment.

One can wander why such approaches have only been developed so late with regards to information related to elements and their species. It is most often stated that regulations need to be in place so that metal species are definitely considered as a primary source of information. Instrument development (hyphenated techniques) and sample preparation have made tremendous progresses with regards to simplification, cost-efficiency and possibility of routine operation. This first aspect should already contribute to facilitate the wider use of metal species assessment in general. More important now is the new enforcement of the REACH (Registration, Evaluation and Authorisation of CHemicals) European legislation. This legislation will require that all new chemical species will require to be evaluated prior introduction in industrial applications. The toxicity of the products will also have to be assessed. This legislation will certainly profoundly promote and motivate development around metal speciation. Indeed in the future, the toxicity of chemical compounds will be assessed via QSAR (Quantitative Structure- Activity Relationship). We have to also process all metal species through the same approach bringing to evidence that metal species of a same element do not have the same reactivity, toxicity and chemical properties. We will have to put into perspective and demonstrate via molecular modelling what we already intrinsically know. It will also be of paramount important to understand the fate and pathways of elements in the environment since we will then take into account the different physico-chemical properties of the elemental species and hence be in a position to model on a global scale their translocation between the different compartments of the environment.

We will present a comparative assessment of the use of QSAR approach and impact on our vision of trace elements species and discuss these implications for trace element speciation in the environment and its application for sustainable growth.

PL 2

The role of trace element speciation in life-science research

Erik H. Larsen

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Selenium remains an element of great importance and also fascination in the study of human health and prevention of disease. The suggested anti-carcinogenic role of this element has attracted much attention from many scientific communities. In order to study the fate of selenium administered as selenised yeast, a pilot study of the planned Prevention of Cancer by Intervention by Selenium (PRECISE) trial was undertaken. A group of 96 elderly Danes (60-74 y) volunteered for a 5-year Se-yeast supplementation pilot study of the trial. The participants were randomised to supplementation with 100, 200 or 300 µg Se/day as selenised yeast or placebo. Following the termination of the supplementation period, the Se contents in toenails, whole blood and in blood plasma were analysed. The scope of the study was to investigate whether the concentration of selenium in toenails was useful as a biomarker of exposure to supplemented selenium. Furthermore, the scope was to understand if toenail selenium ranked the volunteers in the same way, as did plasma or whole blood selenium. The selenium content in biological materials was determined as 78Se by ICP-MS. Selenium-containing plasma proteins including selenoprotein-P (Sel-P) were separated using a heparin affinity column and detected on-line by ICP-MS. The results showed that the selenium concentrations in plasma, whole blood and in toenails (used as biomarkers) were significantly different ($p<0,001$) between the three dosages used and placebo. Furthermore, the expression of glutathioneperoxidase (GPx) and Sel-P showed that GPx was remained constant in plasma from all dosage groups whereas Sel-P increased as function of dosage. This result raises the question, which daily intake of selenium should be recommended from a nutritional and from a disease prevention point of view.

The exposure of humans to nanoparticles (NPs) is a rather new area in life-science and food safety research. Initial experiments using field flow fractionation (FFF) with static and dynamic light scattering (DLS) detection were undertaken. The results showed that gold NPs were efficiently separated by FFF and that the sizes were accurately determined by DLS. Another food-relevant application is the determination clay NPs migrated from clay-enforced biodegradable plastic. The FFF separation of the non-spherical clay poses problems in terms of their separation and their size determination. This will be discussed in detail. Future coupling of FFF with ICP-MS will pave the road towards multivariable detection of the presence of clay NPs migrated into food. Furthermore, it will enable a highly selective detection and therefore superior performance to the light scattering methods currently used.

PL 3

Hyphenated isotope dilution ICP-MS as an accurate tool for speciation analysis

Klaus G. Heumann, Natascha Demuth, Jens Heilmann, Nataliya Poperechna and Lothar Rottmann

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In the past, isotope dilution mass spectrometry (IDMS) was often used for sensitive and accurate determinations of trace elements in different matrices. IDMS is internationally accepted as an accurate analytical method for trace element determinations. Since the first species analyses in 1994 by isotope dilution ICP-MS (1,2) many other investigations in elemental speciation have been carried out with the isotope dilution technique as recently an extended review on elemental speciation has documented (3). Species-specific and species-unspecific ICP-IDMS methods have been established (4), where for the first mentioned technique an isotope-labeled spike of the analyte must be used whereas for the second one any isotope-labeled compound of the element of interest can be applied after separation of the corresponding species (post column isotope dilution technique). Species-specific ICP-IDMS can also be used to validate sample preparation techniques for possible species transformations, a problem which often occurs in species analysis but cannot easily be identified by other analytical methods. This will be demonstrated by a monomethyl mercury transformation during sample pretreatment (5). However, also multiple-spiking is possible which allows to follow interconversion pathways of elemental species (6). Although the accuracy of analytical results of elemental speciation is an important task today, application of hyphenated ICP-IDMS has not become a routine method, up until now, even this technique has the best potential to get it (7).

Coupling of ICP-IDMS with separation techniques like high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and gel electrophoresis (GE), the last-mentioned technique was recently developed in our laboratory by Bettmer and Brüchert (8), allows the determination of elemental species for many different applications covering especially environmental, biological and clinical aspects. Hyphenated species-specific and species-unspecific ICP-IDMS methods allow accurate and relatively fast "real-time" quantification of well known structurally exactly defined but also of unknown elemental species in a chromatogram or electropherogram. Examples for HPLC/ICP-IDMS and GE/ICP-IDMS, including humic substance metal complexes and metalloproteins, and GC/ICP-IDMS analyses for sulfur speciation in petroleum products will be presented. Also multi-species determinations of toxic compounds like monomethyl mercury and butyltins in seafood samples is an actual analytical problem which can properly be solved by species-specific GC/ICP-IDMS (9).

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PL 4

Combining in-situ measurements with speciation techniques to characterize biogeochemical processes in sediments

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Metals play a central role in the biogeochemical cycling of elements in freshwater and marine environments. In sediments, most processes responsible for the transformation of metals occur near redox interfaces, usually within millimeters to centimeters from the sediment-water interface. As a result, analytical techniques developed to analyze metal speciation in these environments must be able to provide information with at least one millimeter resolution. Mercury voltammetric microelectrodes have proven very useful in gaining insight into the speciation of metals in these environments. These microelectrodes provide fast responses, display low memory effects, are capable of detecting many chemical species involved in the transformation of trace metals with good sensitivities (e.g., $O_{2(aq)}$, ΣH_2S, Mn^{2+}, and Fe^{2+}), and display some speciation capability (i.e., soluble organic-Fe(III) and $FeS_{(aq)}$ complexes) which is very useful in characterizing biogeochemical processes in most natural and impacted sediments. Using mercury-gold (Au/Hg) voltammetric microelectrodes deployed in situ from benthic landers and other platforms, we are currently investigating the biogeochemical processes responsible for the transformation of iron, manganese, and sulfur, and their role in the diagenetic processes of carbon in marine sediments. Simultaneously, we are using Au/Hg voltammetric microelectrodes as supporting tools to investigate the role of biogeochemical processes on the transformation of arsenic and uranium in freshwater environments. I will provide examples of these studies and focus on the existence and characterization of soluble organic-Fe(III) complexes in marine sediments.

PL 5

MS-based proteomics turns quantitative: The great future of HPLC-ICP-MS speciation techniques for this challenge

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Mass Spectrometry has become one of the most powerful analytical tools to characterise proteins. As proteomics itself, however, new advances and results in this field have been mostly qualitative. Recent approaches to obtain quantitative proteomic information exist, but they are "relative" because they rely on comparing the signals from a given peptide from two different experiments (usually using stable isotopes for labeling). Absolute quantification (only one experiment) has been scarcely addressed in proteomics. Elemental detection by ICP-MS after HPLC separation, typically used in trace element speciation, may provide a means to modern biochemists for absolute quantitative proteomics, particularly for the study of post-translational modifications (PTMs) of proteins.

For instance, it is known that abnormal Tf isoforms, commonly referred to as carbohydrate-deficient-transferrins (CDTs), are excellent biochemical markers for congenital disorders of glycosylation (CDG) and also for chronic alcohol consumption. We have investigated the usefulness of typical "iron speciation" strategies to develop a method of enough resolution and sensitivity to unable the determination of individual Tf–Fe glycoforms. The method is based on high performance liquid chromatography (HPLC) coupled on-line with ICP-MS to determine ^{56}Fe and ^{57}Fe. This allowed the straightforward detection of six Tf glycoforms in healthy human serum after adequate iron saturation. Intact serum Tf glycoforms analysis by MALDI-TOF and by ES-Q-TOF were used for identification, but isotope dilution analysis using 57Fe isotope will be described for absolute and accurate Fe-Tf isoforms determinations as biomarkers of more common parameters related to alcoholism and imbalances of iron homeostasis.

Another most important PTM of proteins, phosphorylation, determines the activity, subcellular localization, signalling potential, turnover and interactions of a given protein with other proteins, DNA or bioligands. Thus, phosphoproteomics is now an extense and active field of research demanding new ideas for phosphoprotein or phosphopeptide quantifications. The use of element-specific ICP-MS detection of phosphorus and capillary HPLC-ICP-MS analysis of proteins tryptic digests will be described. The high accuracy (around 2%) and precision attained using just a single reference phosphorous containing compound [Bis(4-nitrophenyl) phosphate] for calibration of all phosphopeptides of a tryptic digest of the protein make this strategy ideal for the investigation of small quantitative protein changes in functional and temporal studies involving signalling via phosphorylations, as it will be demonstrated for caseins (1).

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OP 1.1

Monitoring of chromium species and eleven selected metals in emission and immission of airborne environment

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Special aspects of direct emission of a foundry and immission to very closed living areas are studied. Beside the determination of ten heavy metals, Cr speciation was carried out in order to determine the Cr(VI) concentration. With respect to toxicity Cr(VI) species are playing an important role. Human studies have clearly established that inhaled Cr(VI) is a human carcinogen, resulting in an increased risk of lung cancer.

Sampling took place on different days during a period of six weeks. In this period the foundry was busy with the activities of welding and founding in two different halls. It was guaranteed that normal working activities took place in the foundry during all sampling periods. The samples were taken as industrial exhaust directly by the outlet and as airborne sample in the environment with distances between some hundred meters and 2 km from the industrial factory.

Two methods of sampling, sample pre-treatment and mass spectrometric measurement were developed and applied in this study.

- Cr(VI) sampling took place in special impinger systems by absorbing air particles in a buffer of Na_2CO_3 / $NaHCO_3$. A procedure of selective complexation and extraction was developed.
  The extracts were measured by high resolution inductively coupled plasma mass spectrometry (HR-ICPMS).
- Airborne particulate matter was sampled on quartz-filters After digestion with aqua regia several elements (Al, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, and Zn) were analysed as total-element concentration by quadrupole (Q-) ICPMS.

The obtained analytical data are the basis of an indirect exposure study with respect to people who are living in the next surrounding of the foundry.

OP 1.2

Bioremediation of selenium containing waters: Importance of selenium speciation analysis

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The importance of selenium in environmental research is related to the fact that it shows only a marginal line between the nutritious optimum (as an essential element) and toxic effects upon exposure. The environmental fate of selenium compounds varies greatly dependent on its speciation. A huge variety of both anthropogenic and geogenic aqueous waste streams call for an efficient low-tech cleanup solution. Compared to adsorptive or precipitative techniques, the bioreduction of selenate to elemental selenium is a promising, as combining a separation and a detoxification step.

Anaerobic granular sludge previously tested towards selenium bioreduction (1,2) was used to inoculate two continuous Upstream Anaerobic Sludge Blanket (UASB) Reactors with a working volume of 0.46 L, operated at a superficial upflow velocity of 1 m h-1 and a hydraulic retention time of 6 h. The reactors were operated under methanogenic and sulfate reducing conditions using lactate as carbon source at an organic loading rate of 5 g COD L-1 d-1. Selenate was feed at a concentration of 10 µM.

It was shown that most selenate was indeed converted to elemental selenium (3), but still the removal efficiency for dissolved selenium was lower in comparison with selenate removal. During bioreduction of selenate not only elemental selenium can be formed, but also different other selenium species that might show an even higher toxicity (4). Consequently selenium was analyzed species specifically in gas phase (SPME-GC-MS), liquid phase (Ion-Chromatography) and solid phase (differential XRD and µ-XANES). Dimethylselenide and Dimethyldiselenide, two toxic substances, were contributing to these dissolved species, as they were detected using SPME-GC-MS. The contribution of the latter species would not have been revealed by standard methods for selenium analysis (5), clearly underlining the importance of species-specific selenium analysis.

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OP 1.3

A general strategy for monomethylmercury analysis: From low to highly polluted sediments

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The ecological and human health effects of mercury are generally related to the environmental transformations of inorganic mercury to the toxic and biomagnification-prone compound monomethyl mercury (MMHg). The methylation and demethylation processes preferably seem to occur in sediments. Therefore, the accurate and precise determination of MMHg in sediments is the key point to better understanding the biogeochemical cycling of this contaminant and to estimate the associated exposures.

The most critical compartments for speciation are still linked to the solid phase-biota and soil and/or sediments. There are traditional problems related to the extraction; basically, an adequate recovery should be obtained while the species distribution is kept. In soil and sediments the most critical is the very low concentration of organomercury species which are in presence of very high concentrations of inorganic mercury; in general, MMHg does not exceed 1.5 % of the total mercury content in sediments. Furthermore, there are problems in the chromatographic separation and also artificial MMHg generation is nowadays a matter of serious concern.

Thus, the aim of this work has been to develop a general strategy for real sediment analysis. Experiments have been carried out by using spiked natural sediments and certified reference materials. Preconcentration and cleaning procedures have been developed for both low and high polluted sediments specifically to detect low concentrations and to control artefact generation. Mercury species have been acid-extracted with 6M nitric acid by using closed vessel microwave-assisted extraction at the maximum extraction yield. In spite of significant benefits on offer in terms of time, efficiency, solvent consumption and minimum risk of losing volatile compounds, only a few applications have been done using this extraction technique.

The determination has been carried out by using the advantageous and underexploited coupling of capillary gas chromatography and atomic fluorescence spectrometry detection (CGC-pyro-AFS). The separation has been performed after conversion of MMHg into a peralkylated volatile compound. The stability of mercury species under different conditions has been checked. In presence of high inorganic mercury levels, spurious generation of MMHg (below 1%) has been found. Therefore, a cleaning procedure before derivatization (organic solvent extraction with methylene chloride in hydrochloric acid medium) is necessary in order to reduce inorganic mercury from the extract when inorganic mercury concentration is up to 500 ng g-1. Meanwhile, a preconcentration step by nitrogen stream, which makes possible to achieve limits of detection of 2.6 ng g-1 for MMHg (as Hg), is generally required because the analytical concentrations of MMHg in most sediments are very low. The final conditions have been validated by the analysis of two certified reference materials displaying very different mercury species concentration levels, IAEA-405 and ERM-CC-580, with satisfactory results.

OP 1.4

High priority contaminants of the aquatic environment: Speciation analysis for detection and the evaluation of removal strategies

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With the introduction of the water framework directive 2000/60/EC (WFD) the EU aimed at harmonized, uniform and high quality goals for surface water bodies with respect to organic and inorganic micropollutants. A list of so-called priority substances which comprises mainly persistent organic pollutants (POP) and several inorganic pollutants (Cd, Hg, Ni, Pb) was issued. In this context organotin compounds and brominated flame retardants represent a class of substances which affords highly sensitive and selective methods for detection and accurate quantification in different environmental compartments. We will discuss the figures of merit of different separation methods in combination with mass spectrometric detection and their applicability for investigation of the fate of these substances in the aquatic environment.

It is evident that the contamination of the aquatic environment can be avoided by fit-for-purpose elimination strategies. We have assessed the potential of ionic liquids for removal of organotin compounds, brominated flame retardants and priority metals from waste water of different contamination sources employing the above mentioned techniques. First data will be presented.

OP 1.5

Environmental speciation analysis: A practical viewpoint and some applications in a contract laboratory

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The development of analytical techniques for the determination of chemical species has been one of the growing features of the 1990's in analytical chemistry. The determination of these chemical species, known as speciation analysis, is currently performed routinely in many laboratories to control the quality of the environment. Some typical examples are: tributyltin in water and sediment rather than simply determining total tin, arsenic species in drinking and ground waters), methylmercury in fish, selenium in yeast, wheat flour and tap water, chromium species (e.g. Cr(VI) in the workplace, environmental risk assessment (e.g. arsenic and chromium species in polluted soils).

Speciation has been considered in a few EC Directives. Nevertheless, the need for determination of chemical species is well established and today laboratories have to manage with this goal and have to propose a panel of fit for purpose speciation analysis related to their scope of activity. The determination of the total metal concentrations is not always the appropriate response to address the problem because we clearly know that it is rather the chemical forms of an element which controls its fate, impact and behaviour. Consequently, we have developed rapid and robust speciation measurement methods with an emphasis on retrieving as much information as possible from the analysis with a minimum of sample pre-treatment. (There is always a danger that sample pre-treatment can alter the speciation.)

Elements most often investigated in routine analysis by our laboratory are organotin compounds in wide range of environmental samples; arsenic species in polluted soil; ground water and leachates from soil samples; chromium VI in soil and sludge samples and also selenium species in drinking water samples, see references (1-6). Some practical examples will be given to illustrate and highlight the problems encountered with speciation analysis as well as ensuring suitable Quality Assurance and Quality Control (QC/QA) protocols required to achieve ISO 17025 speciation accreditation.

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OP 1.6

Chemical speciation fingerprints by geochemical modelling after the characterisation of the leaching behaviour of various materials

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The characterisation of materials, such as soil, contaminated soil, sediments, sludge, compost, wood, waste and construction products, is entering a new phase with the latest developments in characterisation leaching tests and associated chemical speciation modelling capabilities. The increased insight in contaminant release controlling processes shows that the release of contaminants from materials largely depends on the chemical speciation of constituents. Therefore, geochemical reaction/transport modelling forms the basis for prediction of long term release behaviour and chemical partitioning of elements between different chemical binding forms (chemical speciation fingerprint). Using proper thermodynamic stability data and other solubility controlling parameters allows quantification of binding to Fe-oxide, Al-oxide, dissolved organic carbon and particulate organic matter and minerals. For geochemical speciation/ transport, the modelling framework ORCHESTRA (Objects Representing CHEmical Speciation and TRAnsport models) (Meeussen, 2003) coupled to LeachXS as the data management system is used.

Proper waste management, satisfying environmental and health related criteria, requires a fair amount of information on a wide range of materials/substances. This information involves a proper material description, composition data, physical properties of a material, data on leaching of inorganic and organic constituents and, whenever relevant, biological properties. Release of constituents through leaching is a key aspect for judging environmental impact of material management options focussed on beneficial applications as well as on disposal, because most pathways of transport go in one way or another through a dissolution phase. The database/expert system (LeachXS) has been developed to facilitate data retrieval, test comparison, geochemical modelling and scenario evaluation using a full mechanistic description of processes.

The pH dependent leaching behaviour in combination with the calculated chemical speciation of constituents in the solid phase and in solution allows judgement of the bioavailability of constituents through their distribution between the free and DOC-bound fraction in solution. For judgement of treatment options, this means of assessing changes in material behaviour is potentially very powerful to make the right choices. For impact assessment this approach allows far better and more realistic predictions of environmental impact than the use of a constant factor that describes the distribution of contaminants between the liquid and solid phase (“Kd concept”).

OP 1.7

Simultaneous speciation of As, Se, and Cr: Feasibility for real samples

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An analytical method has been developed that allows the simultaneous determination of As(III), As(V), Se(IV), Se(VI), Cr(III), and Cr(VI) species using high performance liquid chromatography (HPLC) separation with ICP-MS detection. In order to reduce interferences for the determination of As, Cr, and Se by ICP-MS, a Dynamic Reaction Cell (DRC) ICP-MS system was used to detect the species eluted from the chromatographic column. A variety of reaction cell gases and conditions may be utilized and the advantages and limitations of the gases tested will be presented and discussed.

By complexing the Cr(III) with EDTA prior to injection into the chromatographic column, the method allows the separation and detection of the As, Cr, and Se species of interest using the same chromatographic conditions in less than 2 minutes (figure 1). The robustness of the method to concomitant element and anion effects will be discussed as well as the method's applicability for the analysis of environmental and geological samples including waters, soil leachates and simulated bio-fluid leachates. The method uses relatively inexpensive 3 cm C8 columns and a tetrabutylammonium hydroxide/EDTA/methanol mobile phase.

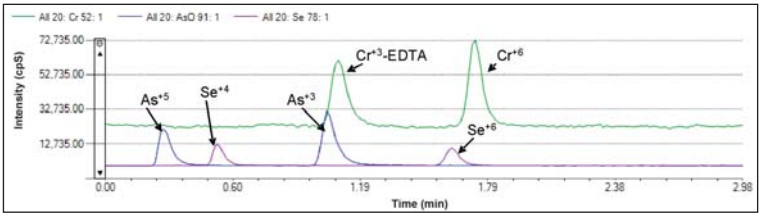

Figure 1. Example chromatogram of 20 ppb As^{+3}, As^{+5}, Se^{+4}, Se^{+6}, Cr^{+3} (as EDTA complex), and Cr^{+6} using a single set of chromatographic conditions and oxygen as the reaction gas in a DRC-ICP-MS. Using oxygen as the reaction gas, arsenic is measured as AsO at mass 91

OP 1.8

The determination of PBDEs by GC-ICP-Q-MS

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Polybrominated diphenyl ethers (PBDE) are flame retardant chemicals used in everyday household items such as computer casings, televisions and household textiles. Leaching and migration of these chemicals from domestic products has been reported as the cause for the widespread presence of these compounds in our environment. Coupled with their persistent and accumulative nature, PBDEs tend to build up in the fat tissue of living organisms. Accumulation and toxicity issues of PBDEs have stimulated legislation where for example, the EU directive 2003/11/EC prohibits the use of penta-BDE and octa-BDE for the member states of the European community and sub-ppt annual average levels of penta-BDE is the recent policy for inland waters in the EU Water Framework Directive (WFD).

PBDEs are predominantly analysed by GC-NCI-MS, GC-HRMS or GC-ECD. This paper investigates the potential of GC-ICP-Q-MS for the determination of PBDEs. Several analytical considerations such as GC column and injector type are discussed and analytical figures of merit are presented and compared to some typical values from alternative instrumental approaches. The application of this methodology for the analysis of PBDEs in NIST SRM 2977 Mussel Tissue and NIST 1941b Marine Sediment is also presented.

OP 2.1

Microwave-assisted enzymatic hydrolysis: Evaluation of its capabilities for Se-speciation

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Selenium (Se) is an essential nutrient to humans, animals and micro-organisms, playing an important role in maintaining a healthy immune system, fertility and thyroid metabolism. However, nutritional bioavailability and toxicity are dependent upon its chemical form and concentration. The accurate determination of Se species requires extraction methods that exhibit high extraction efficiencies, and are capable to preserve the identity of the Se-compounds.

Enzymatic hydrolysis, utilising protease XIV or proteinase K, has been the most widely used technique to release protein-bound compound such as selenomethionine (SeMet) in a number of food/supplement matrices (1). Efficiency of SeMet extraction with proteolytic enzymes has been found critically dependent upon incubation or extraction time and sample to enzyme ratio. Successful enzymatic approaches involve the use of multiple consecutive steps, with duration of at least 18 hours each (1). Such tedious multi-step approaches have often resulted in transformation and/or interconversion of target Se species (e.g. SeMet).

More recently, the combination of enzymatic digestion with probe sonication has been reported as a promising tool for extraction of Se species from food and supplements, overcoming the main drawback of the traditional enzymatic treatments (e.g. incubation and bath sonication) such as long sample treatment times (2). It has proven to be a powerful system in order to speed the extraction of Se species from yeast, oyster and mussel tissues, but, of course, the extraction efficiency was found to be critically dependent on the extraction conditions. Preliminary work in our laboratory has shown, for the first time, the promising capabilities of enzymatic extraction assisted by microwave energy for enhancing the enzyme activity without varying the nature of target dietary Se species. To the best of the authors's knowledge, the use of enzymes with microwave energy for Se speciation studies has never been reported.

This work aims at evaluating the potential of microwave-assisted enzymatic hydrolysis, in comparison with conventional enzymatic hybridization, for the extraction of SeMet from food samples, followed by HPLC-ICP-MS analysis. The influence of different parameters such as extraction time, enzyme type, solvent volume and microwave power on the extraction efficiency of SeMet was investigated. The use of enzymatic extraction assisted by microwave energy led to SeMet extraction efficiencies, which are similar to those obtained by conventional multi-step enzymatic hydrolysis. However, with the newly developed approach, the extraction time was found substantially shortened. In addition to this, the simplicity and robustness of the method offers a straightforward approach that can be applied in future to the investigation of Se species distribution in other complex matrix samples.

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OP 2.2

Stabilization of As-species in water using different SPE materials

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Arsenic in groundwater has become a serious problem particularly in India and Bangladesh (1). However, groundwater of former industrial areas can also contain remarkable amounts of arsenic, especially in Eastern Germany. As the exposure of the general population to arsenic occurs mainly through arsenic in drinking water and food, a reliable method to detect and eliminate arsenic from water is required. The water analysed in this work contains higher amounts of sulphur, iron and miscellaneous organic compounds beside the different investigated arsenic species, arsenous acid (As (III)), arsenic acid (As (V)), monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA). This matrix poses a challenge for the stabilisation of the arsenic species.

As the common additives like EDTA (2) and phosphoric acid (3) could not prevent the species from conversion several materials for solid phase extraction (SPE) were tested for their ability to adsorb the different arsenic species. The behaviour of As(III), As(V), MMA, DMA and Arsenobetaine (AsB) towards different ion exchange materials were investigated. AsB can not be found in the water samples but as it is a main component in fish and mussels (4) it was also included in the investigations. A strong anion exchanger was used to adsorb As(V), MMA and DMA and to stabilise the arsenic species which are mainly present in anionic form. The possibility of selective elution according to the pKa of the different acidic species was investigated. The As concentration in particular fractions was determined by ICP-MS and HPLC ICP-MS.

In acidic solution, AsB could be adsorb on a strong cation exchanger and eluted with 0.1 M NaOH. As AsB is an arsenoorganic acid with a pKa of 2.2 and has a permanent positive charge on arsenic, the compound can be transferred to a cation by acids and adsorb on a cation exchanger. For elution the compound is transferred to a zwitterion by a base.

The influence of different matrices on SPE was investigated. Model waters were created that contained high amounts of either sulphur or iron or both sulphur and iron. Also the influence of different salinity of the water was investigated to check if the method can also be used for marine samples. For the determination of arsenic species in fish it is important to know the influence of the fat matrix on the adsorption capability of the ion exchanger. So various amounts of fish oil were added to the solutions of the arsenic compounds and their influence on SPE determined.

At last real fish and water samples were investigated and the results of SPE and HPLC separations were compared.

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OP 2.3

Arsenic accumulation and speciation in freshwater fish living in arsenic-contaminated waters

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Striped snakehead (Channa striata), carnivorous freshwater fish that serve as popular food in Thailand, were collected from a reference site (1.4 µg As L^{-1}) and from two arsenic-contaminated ponds (Pond A 550 µg As L^{-1} and Pond B 990 µg As L^{-1}) in southern Thailand and analysed for arsenic by inductively coupled plasma mass spectrometry (ICPMS) and for arsenic species by HPLC/ICPMS performed on aqueous methanol extracts of muscle, liver and gill (n=3 fish from each site). Mean total arsenic concentration in muscle tissue of Channa striata collected from the reference site was 1.9 µg As g^{-1} (dry mass) while fish from the contaminated sites contained 13.1 µg As g^{-1} (Pond A) and 22.2 µg As g^{-1} (Pond B). Liver and gill tissues showed similar increasing arsenic concentrations on going from the reference site to Ponds A and B, with Pond B showing the highest levels. Speciation analysis on the three tissues showed that, although arsenate was the major extractable arsenical in reference fish (e.g. 0.73 µg As g^{-1} in muscle tissue), dimethylarsinate was by far the dominant arsenic species in fish from the two contaminated sites. The study shows for the first time a clear effect of water arsenic concentrations on natural fish tissue arsenic concentrations, and is the first report of a freshwater fish species attaining arsenic concentrations comparable with those found in marine fish species. Furthermore, the high concentrations of toxic inorganic arsenic (predominantly arsenate) in the muscle tissue of the edible fish Channa striata have human health implications and warrant wider investigations.

LC/ESI-MS study of the structure of germanium sesquioxide (Ge-132) in aqueous solution

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Organogermanium compounds have elicited increasing interest in recent years: They are becoming more and more popular as medication or nutritional additive due to their proven or assumed beneficial effects to human health. They are said to act as effective chemopreventive agents against certain forms of cancer or autoimmune diseases such as AIDS (1,2). At the same time, critical reports warn against the uncontrolled use of organogermanium compounds as nutritional additives (3,4). These contradictory reports call for effective analytical methods for the monitoring of organogermanium compounds contents in nutritional additives.

Only few methods are reported for the analysis of organogermanium compounds, and in particular the most commonly used Germanium sesquioxide or Ge-132. These rely either on total element determination, or on the element specific (or even unspecific) detection of the organogermanium after chromatographic separation (5,6). This leaves the true nature of the Germanium sesquioxide in aqueous solution unrevealed.

For this reason, an LC-electrospray-MS study was undertaken to detect the structure of the Germanium species actually present in aqueous solutions of Germanium sesquioxide. Measurements were performed in direct infusion mode to avoid loss of Ge species on the chromatographic column with negative ion electrospray-MS detection. Spectra of Ge species are highly depending on the measurement conditions. They typically feature an intense molecular peak at low fragmentor voltage which is accompanied by several other signals. These signal clusters – easily identifiable as Ge-containing due to the characteristic isotopic fingerprint of Ge – are found both at higher masses, indicating the presence of (intra-/intermolecular) condensation products and at lower masses (from characteristic fragments). LC-ESI/MS provides thus valuable information on the form of Ge species actually present in aqueous solution. In addition to this, LC-ESI/MS can also be used to study the complexation of Ge sesquioxide by low-molecular weight organic compounds. This may be an important step in the elucidation of possible action mechanisms of Ge-132 in the human body.

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Gas chromatography coupled to microwave-induced plasma emission detection (GC-MIP-PED) for the speciation analysis of mercury

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The plasma emission detector (PED) is based on a microwave induced plasma source (MIP) with optical emission detection. Hyphenated to a GC, the system allows for fast and robust detection of mercury for speciation analysis. In contrast to other MIP systems, the presented plasma source does not require complex water cooling. Moreover, the plasma source can cope with high loads of organic solvents.

Wavelength separation is achieved with an interference filter, oscillating in the light path between the plasma and the photo diode. This filter transmits only a small bandpass around the atomic mercury emission line at 253.652 nm. Its main characteristic is that tilting towards incoming radiation results in a shift of the transmission profile to lower wavelengths. By oscillation of the filter and modulation of the signal readout, an efficient background correction is achieved.

The advantage of the PED, compared to inductively coupled plasmas (ICP) is the low noble gas consumption. ICPs usually require around 15 L min^{-1} of argon, whereas the PED is able to create a stable plasma discharge with less than 0.1 L min^{-1} of helium.

The set-up of the system will be presented as well as analytical figures of merit. The detection limit reaches down to 1 pg Hg (absolute) in combination with a selectivity for mercury against carbon exceeding 10^6. The data show that selective and sensitive detection of mercury species can be realised with this system. As first samples, the results of the analyses of fish tissue shall be presented.

OP 2.6

Determination of Se species in Se enriched and drought exposed potatoes by HPLC-ICP-MS and HPLC-UV-HG-AFS

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Selenium (Se) is an important microelement for humans and is toxic for humans at high doses. It plays a role in the prevention of atherosclerosis, specific cancers, arthritis, and altered immunological functions. Cultivation of plants enriched with Se could be an effective way of producing Se rich foodstuffs, with benefits to health. The beneficial effects of Se are dependent on the chemical form, selenomethionine (SeMet) being the most readily assimilated form. The aim of this work was to study Se accumulation and to identify the Se species in potato (*Solanum tuberosum* L.) cultivar Desiree in Ljubljana, Slovenia, enriched in Se by foliar fertilisation. Four combinations of treatments were conduced: well-watered plants with and without Se foliar spraying, and drought exposed plants with and without Se foliar spraying. Potato was foliar spraying with aqua solution containing 10 mg Se per L in the form of sodium selenate. The Se content was found to be less than 117 ng/g in non treated and in the range 300-1000 ng/g in Se treated potato. Beside the total content of Se, the chemical form in which Se is present is almost most important due to the differences in bioavailability and toxicity. Water-soluble Se compounds were extracted from samples by water, than by enzymatic hydrolysis with enzyme protease XIV, amylase and combination of protease XIV and amylase. Separation of Se species ($SeCys_2$, SeMet, SeMeSeCys, selenite and selenate) was made by anion exchange column (Hamilton PRP-X100) and a cation exchange column (Zorbax 300-SCX). Detection was performed by two techniques, HPLC-ICP-MS and HPLC-UV-HG-AFS. SeMet and selenate were the main species in potato, regardless of the growth conditions. Concentration of selenate was independent, while concentration of SeMet changed using different extraction techniques. The greatest amount of SeMet (40 %) was defined in extracts obtained with enzymatic hydrolysis, using combination of protease XIV and amylase. In water extracts we obtained about 60 % and in enzymatic extracts about 80 % of identified Se compounds. For major compounds comparable results were obtained by both techniques, while some unknown compounds in trace level were observed using HPLC-ICP-MS.

OP 2.7

Determination of methylmercury in fish from the marine and freshwater environments in Ghana using gas liquid chromatography with electron capture detection technique

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Concentrations of methylmercury residues were determined in different marine and freshwater fishes from Ghana. Samples were treated with ethanolic potassium hydroxide in water bath at 100o C for 1 h. After neutralizing with HCl and washing with hexane, methylmercury was extracted with dithizone in toluene, cleaned up and determined by Gas Liquid Chromatography with Electron Capture Detection. The method was sensitive with good precision (std dev=1.5 - 1.6), detection limit of 5 ng g^{-1} and provided good separation for organomercury compounds. The method was applied to certified reference material and different fish samples. A total of forty four (44) samples covering twenty six (26) species of fish were analysed for methylmercury using the method. Concentration of methylmercury in the edible muscle tissue of the tested fish ranged from 7.13 to 106.77 ng g^{-1} wet weight. Methylmercury was the dominant form of mercury in the samples with percentages between 90 and 106 % of the total mercury. The concentrations of methylmercury in the fish samples obtained do not however constitute any significant mercury exposure to the general population through fish consumption.

Comparison of different numerical approaches for multiple spiking species-specific isotope dilution analysis exemplified by the determination of butyltin species in sediments

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The application of isotope dilution analysis (IDA) in elemental speciation has allowed in the past years the development of highly accurate and precise quantification approaches for the determination of a wide range of elemental species even when analysing complicated matrices. Those applications have been always performed under two different spiking modes: species-specific and species-unspecific spiking. Nevertheless, only the first can be regarded as a reference or highly qualified primary method for speciation analysis as the unspecific mode is exclusively limited to the correction of errors derived from the detection step.

The main disadvantage of the species-specific spiking is the need of spike solutions containing the species to be analysed in an isotopically labelled form (previously synthesised or acquired if they are commercially available). However, once a complete mixing between the added enriched and the endogenous species is achieved, all the traditional advantages of isotope dilution analysis can be fully exploited. Furthermore, when several species of the same element need to be analysed, each compound can be enriched in a different isotope of the element, opening up a unique capability of quantification: Multiple Spiking Species-Specific-IDA. This form of isotopic labelling is able to quantify the concentration of species even when opposite or simultaneous degradation processes occur. Therefore, the multiple spiking is normally used for two main purposes: first, to study the extent or rate of transformation processes of elemental species in natural ecosystems or living organisms and second, to correct for the transformation reactions that the analyte species may suffer during the chemical analysis.

Depending on the availability of the isotopically enriched species and the complexity of the interconversion model (number of species to be analysed and their possible degradation/formation pathways), a more or less sophisticated and specific mathematical approach must be developed to quantify the extent of those processes and finally, the species corrected concentrations. An examination and comparison of all the mathematical approaches for multiple spiking species-specific isotope dilution analysis published so far in the literature is presented in this work with the determination of TBT and DBT in sediments. The basis of four different numerical approaches -“Calculation of Stable Isotope Concentrations”, “Speciated Isotope Dilution Analysis”, “Species-Specific Isotope Dilution Analysis” and “Isotope Pattern Deconvolution”) are explained and compared in terms of complexity and analytical figures of merit. The capability of extending the methodologies to a higher number of analytes by the use of additional enriched species as well as the specific advantages of the different methods will be discussed.

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OP 3.2

Beyond GC/ICP-MS: New tools for elemental speciation of volatiles

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The identification of volatile elementorganic compounds is presently performed either by elementspecific or moleculespecific detectors. However, the combination of these complementary detection techniques is most promising.
The presented work proves the technical feasibility of parallel EI-MS and ICP-MS detection after gaschromatographic separation by hyphenation of the respective instruments and shows the conceptual and constructional realisation of this scheme (1).
Synchronized acquisition of both elementspecific and moleculespecific data enables highly sensitive elementspecific quantification and monitoring of moleculespecific data for qualitative verification at the same time. Moreover, the interpretation of complex EI-MS spectra is simplified because the correlation of element information with the signal at a certain retention time facilitates the discovery of chromatographic peaks.
Selected studies demonstrate the applicability of this new analytical tool for various tasks in trace element speciation as low detection limits and high linearity - the benefits of ICP-MS technology – are sustained.

Furthermore, first results will be presented using sector field HR-ICP-MS for detection of GC separated volatiles. Such a system offers outstanding sensitivity for ultra trace speciation in low resolution mode (e.g for environmental monitoring of mercury species) and highly elementspecific detection nearly free from isobaric interferences in medium and high resolution mode (e.g. for speciation of sulphur in beverages or fuel).

The benefits of the presented approaches for elemental speciation of volatile analytes in terms of quality assurance are highlighted:
Misinterpretation of chromatographic data can be avoided and the reliability of results (e.g. identification of unknown species) is improved.

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Cryotrapping-cryofocussing-GC-ICP-MS (CT-CF-GC-ICP-MS) analysis of microbial volatilized selenium sorbed on soil minerals

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Selenium has major nutritional and biological function but the range between essential and toxic concentration is very small. The more toxic Se(IV) and Se(VI) are water soluble and hence bioavailable. The iron oxide and clay content of soils can affect the bioavailability through adsorption reactions, whereby Se(IV) is stronger adsorbed than Se(VI) (1). Microbial methylation is the most reliable natural attenuation process of enriched Se in the environment (2).

In order to investigate the alkylation / methylation process, Alternaria alternata, known as a Se resistant and active methylating saprophytic fungus (3), was used for incubation studies. First we observed the alkylation of dissolved inorganic selenium (SeO_3^{2-}, SeO_4^{2-}) and found different alkylated species over a wide pH range. Then, different selenium enriched materials, e.g. goethite enriched by SeO_3^{2-} or SeO_4^{2-} adsorption, were incubated with *A. alternata* inoculums to study the dependence of solid selenium binding on alkylation reactions. For Se speciation studies we used different analytical tools. Volatile selenium species were measured with a cryotrapping / cryofocussing gaschromatographic system coupled with ICP-MS. Beside the dominantly occuring dimethyl selenide (DMSe) and dimethyl diselenide (DMDSe) we found also other alkylated species like diethyl selenide (DESe) and diethyl diselenide, especially in dissolved selenium experiments. In adsorption experiments the aqueous inorganic selenium species were measured by hydride generation – atomic absorption spectroscopy. In icubation experiments we used a hyphenated HPLC-ICP-MS to separate the dissolved selenium species.

In our incubation studies we found different patterns of alkylation of dissolved and adsorbed selenium. For dissolved Se, Se(IV) were alkylated in higher amounts and we found more volatile species than for Se(VI) caused by a lacking reduction step in the methylation mechanism (4). In the opposite we found a higher methylation for Se(VI) than for Se(IV) adsorbed on soil minerals because of different kinds of adsorption (5).

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OP 3.4

Investigation of non-covalent metal-phytosiderophore species by electrospray-high resolution-mass spectrometry

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The direct analysis of metal species in complex biological samples is a prerequisite for understanding the biological role(s) of these metals in different biosystems. Especially, the analysis of small, non-covalent metal complexes is challenging because the latter can be highly reactive, and there is always a risk of changing metal species equilibria during analytical procedures. Another problem is the unequivocal identification of unknown metal species, which often must be done at very low concentrations and sometimes also in very low volumes, due to the limited amount of isolated biological samples.

Therefore, we developed a method for the direct analysis of small non-covalent complexes based on high-resolution mass spectrometry applying nano-electrospray ionization-Fourier transform ion cyclotron resonance MS (nano-ESI-FTICRMS). The use of FTICRMS not only provides high resolution but also high mass accuracy, enabling the unambiguous determination of elemental compositions. Hence, this enables the unequivocal identification of non-covalent metal chelates.

The applicability of this method is demonstrated by the investigation of the iron uptake and translocation in grasses via phytosiderophores and the non-proteinogenic amino acid nicotianamine (NA). Equilibria of DMA and NA in the presence of Fe(II) and Fe(III) are controversically discussed in the literature, and the relative stability (and thus importance) of different iron species in planta is not clear.

The method enables the direct analysis of NA and DMA complexes with Fe(II) and Fe(III) in one solution without separation. Although the two ligands differ only by one mass unit, and consequently there are pairs of iron species (i.e. Fe(II)-NA and Fe(III)-DMA), which differ only by 0.0239 m/z units, the use of FTICR-MS enables the unequivocal identification of all four iron species (NA-Fe(II), NA-Fe(III), DMA-Fe(II), DMA-Fe(III)) (1). Advantages of the method are the low sample consumption (only some µL of solution are needed), and the possibility to verify the stoichiometry of metal species with high accuracy and to assign the redox state of the chelated iron. Additional work includes the investigation cadmium-phytosiderophore complexes also with respect to ligand exchange prosseses (2).

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CE-ICP-MS as speciation technique to analyze the complexation behavior of lanthanides with humic acid

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For the long-term disposal of radioactive waste, detailed information about geochemical behavior of radioactive and toxic metal ions under environmental conditions is necessary. Humic acid (HA) can play an important role in the immobilisation or mobilisation of metal ions due to complexation and colloid formation. Therefore, we investigate the complexation behavior of selected metal ions (europium and gadolinium as homologues of the actinides americium and curium) with HA.

To guarantee reliable results, two different analytical methods were used to determine stability constants by differentiating between uncomplexed metal ions and metal ions which are bound to HA (1). The evaluation of complex stability constants was performed by applying ultrafiltration, mechanically separating non HA complexed ions of Eu and Gd from the metal-humate complexes. Measurement of the separated ions from the filtrate was done by ICP-MS. As an alternative technique, capillary electrophoresis (CE) was hyphenated with inductively coupled plasma mass spectrometry (ICP-MS). With this method both the uncomplexed metal ions and metal humate complexes can be simultaneous detected in one analysis step.

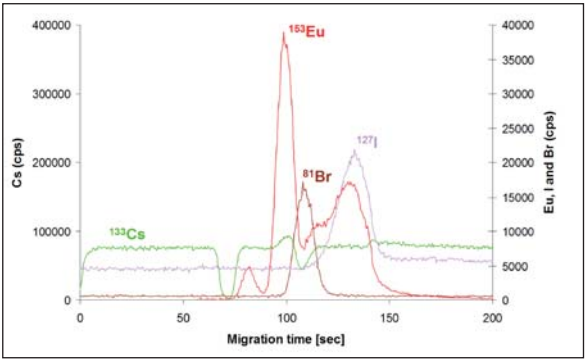

Figure 1. CE-ICP-MS electropherogram showing Eu species (^{153}Eu) complexed or uncomplexd with iodinated HA (^{127}I), 1-bromopropane (^{81}Br) as neutral marker and Cs (^{133}Cs) as CE electrolyte flow marker (1).

Generally, HA are not detectable by ICP-MS. To make the HA visible, we used a chemical procedure to halogenate the aromatic hydrocarbon-rings in the HA with iodine as “ICP-MS” marker. This results in a unambiguous differentiation of ICP-MS metal signals, assigned to HA complexed and uncomplexed metal species. The authors thank the BMWA for financial support of the project through grant no. 02 E 9683.

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CE-DAD-ICP-MS as tool for investigation in the complexation of metals and for metal speciation

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The technique of CE-ICP-MS (1) was enhanced during the recent years by a diode array detector DAD to give the opportunity to determine metal ion species as well as complexes and free ligands. The big advantage is that these three types of analytes can be separated and detected in one run.

The performance of this technique has been improved and the new developments show an optimised sample introduction system to lower the limits of detection. It is now possible to introduce 100% of the sample uptake into the plasma. It is now possible to determine metal species with 1 ppb and humic acid as an interesting ligand with a limit of detection of 20 mg/l.

By the use of the complete spectra of the diode array detector it is possible to assign the determined species. Different examples will be gives that show the prospects.

The technique was also applied to determine complex formation constants. This will at the example of Ho and iodine marked humic acid (figure 1).

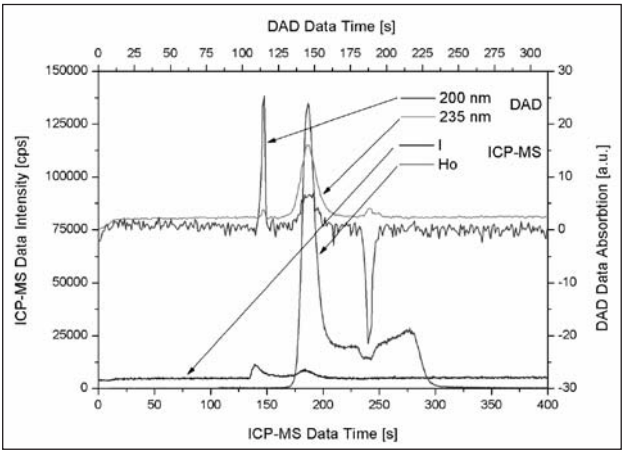

Figure 1. Electropherogram obtained by DAD and ICP-MS detection from a solution containing iodine marked humic acid and Ho.

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OP 3.7

Comparison of mininaturised sample treatment systems for As speciation in human hair and nails

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Arsenic speciation in order to understand its biological pathways, transformation and accumulation/elimination processes is still a hot topic in Analytical Chemistry, since an important part of the worldwide population live in areas which have to be considered moderate to highly contaminated. Nowadays, sensitive and robust separation and detection techniques such as HPLC-ICP/MS are available, wherefore sample treatment usually becomes the limiting step of an analytical method regarding time, sample and reagent consumption and species integrity.

The most commonly used bioindicators to assess human exposure to arsenic are blood, urine, nails and hair. The latter possesses some advantages over the former, in particular that commonly found concentrations are elevated, the matrix is easy to sample, store and to transport. However, methods for arsenic speciation are scarce for these type of matrices, in general these are multi-step treatments which suffer from elevated analyte extraction times.

In this communication, modern approaches making use of miniaturised pressurised liquid extraction (PLE) and ultrasound probe sonication (UPS), where appropriate in combination with mixed enzymatic treatments, applied to arsenic speciation in hair and nails will be critically discussed. Critical parameters during the optimization procedure for the different techniques will be presented and their suitability highlighted by application to real samples coming from highly arsenic contaminated areas in the middle and lower Ganga Plain, India.

Both methods profile as powerful tools for sample treatment, providing a mild procedure to extract biomolecule bound organometallic species, which would be destroyed by more aggressive leaching or solubilization methods. In most cases, quantitative extraction in a very short time with respect to generally used methods could be achieved (reduction from hours to minutes). Other advantages are a simplified sample handling and the reduction of reagent consumption and sample amount.

OP 3.8

Trace element speciation modelling applied to environmental systems: The deceiving reality and some new perspectives

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The use of computer programs for the calculation of the distribution of the species formed in aqueous systems is not new, e.g. a model for seawater was developed more than 40 years ago. However, nowadays the technique is potentially more available due to the advances in computer technology and, at present, speciation modelling can be considered as a routine tool in many fields. This has lead to the false impression that any system can be modelled and computer models are too often used as black boxes. The reality is that the validity of most of the results obtained by applying these models is highly questionable. The result of a survey of recent papers published in some well-known environmental journals will be presented to illustrate this point.

Speciation models have evolved very little over of the years and they present today almost the same limitations as 30 years ago. Some of these limitations, in particular those related to the quality of the equilibrium constant values used and issues associated with the role of natural organic matter, have been largely discussed in the past. More often ignored are the limitations imposed by the lack of adequate analytical methods for the quantification of the mineral and macromolecular ligands present in natural aquatic systems. The new perspectives opened by the recent development of methods that allow the quantification of inorganic colloids (1) and the refractory fraction of natural organic matter (2) will be discussed in this communication.

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OP 4.1

Direct monitoring of metabolic Fe-species equilibria in plants by CV and MS

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Uptake of iron into plants and subsequent metabolic reactions involve changes of the complexing ligand as well as changes of the redox state of iron. In grasses, a particular uptake system for iron is present, based on the excretion of phytosiderophores (PS) and uptake of respective Fe(III)-PS-species by the YS1 transporter, located in the plasmalemma membrane. Up to this point, the respective mechanism is quite well understood. The mechanism of iron release from the very stable Fe(III)-PS-species inside the plant, however, remains to be elucidated. One proposed mechanism involves the reduction of ferric phytosiderophores in the presence of a strong iron(II)-chelator, probably via ternary complex formation. In other words: the ligand exchange reaction is closely linked to a preceding, or even simultaneous, redox reaction of the iron center. In order to investigate such reaction(s), methods are needed, which can directly monitor – in real time, and without disturbing respective equilibria – all relevant species in the system. This is very difficult, if not impossible, by using separation-based analytical speciation methodology (e.g., HPLC, CE).

We present here, for the first time, a direct experimental verification of the redox-mediated conversion of ferric PS species by ascorbate in the presence of the plant ligand nicotianamine (NA), leading to the formation of the ferrous NA-chelate. The reaction is monitored by cyclic voltammetry (CV), and by high resolution mass spectrometry (Fourier transform ion cyclotron resonance mass spectrometry). CV is very sensitive to changes of the chemical composition of redox-active species, which are detected by the corresponding shift in redox potential, and MS enables an unequivocal identification of all educts and products, incl. intermediates. The results are discussed with respect to the most probable reaction mechanism and concentration dependence of the reaction rate, but also consequences for our understanding of the role of metal-PS-species in plant metabolism are highlighted. For example, the reaction rates of three different, but very similar PS were found to be inversely related to the thermodynamic stability of their ferric chelates.

Determination of trace metal speciation parameters by using screen-printed sensors in stripping chronopotentiometry

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Electrochemical techniques, especially stripping methods, are of particular relevance for studies on metal ion speciation. They provide adequate sensitivity and the analytical signals inherently can contain direct speciation information. In particular, these methods are sensitive to the lability which is characterized by a shift of the characteristic potential, and, for unequal diffusion coefficients, by the magnitude of the analytical signal (1). However, lability is an experimentally defined parameter that depends on the effective time scale of the analytical technique. The analytical challenge is therefore to develop a technique able to determine parameters that describe the lability such as the stability, and rates of association and dissociation. The stripping chronopotentiometry (SCP) method appears as the most appropriate for ion speciation studies because it leads to minimize adsorption effects under conditions approaching complete depletion (2). Moreover, Van Leeuwen and Town (2002) have demonstrated that the stripping chronopotentiometry at scanned deposition potential (SSCP) allows a straightforward description of the lability (3). Ideally, such measurement should be made in situ to avoid sampling and sample handling artifacts (4). The purpose of this work has been therefore to develop the SSCP for screen-printed sensors.

First, analytical parameters of SCP have been optimized to approach conditions of complete depletion. This regime has been reached for a stripping current higher than 3 µA. However, it was demonstrated that a stripping current of 10 µA allowed to skip the sample deoxygenating step which is liable for change the pH sample and therefore speciation (5). Then, the adsorption effects on the working surface of the screen printed sensor have been investigated in a Cd solution containing a complexing ligand, the pyridine-2,6-dicarboxylic acid (PDCA). It has been shown that SCP measurements at screen-printed sensors were essentially free from adsorption effects whatever the electrodeposition time used. Finally, the SSCP has been applied to a range of ligand concentrations (from 10^{-4} to 10^{-5} mol L^{-1}). The experimental curves have been successfully compared to those calculated, which allowed to determine the stability constants of the Cd complex. The SSCP at screen-printed sensors appears therefore as a promise tool for the in situ determination of the lability criteria in order to provide the information required to the ecotoxicological risk assessment.

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OP 4.3

AGNES: Determining free Zn concentration in seawater

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The free concentration of metals present in solution is a key datum in environmental studies, as recognised by the Free Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM) which correlate the uptake of the element by the organisms with the concentration of the free metal.

AGNES (Absence of Gradients and Nernstian Equilibrium Stripping), a new electroanalytical technique (1) developed to determine free metal concentration in solution, can be an alternative to other existing techniques, specially in cases, such as Zn, where a commercial ion selective electrode (ISE) is not available. AGNES has been validated against ISE and against Resin Titration (2).

AGNES consists in the application of 2 stages: i) In the deposition stage, we apply a program of potentials aiming at preconcentrating the metal inside the amalgam until the system reaches Nernstian equilibrium with the solution and no concentration gradients in the amalgam or in the solution and ii) in the stripping stage, we determine the concentration of metal reduced in the amalgam. For that purpose, we apply a sufficiently less negative potential producing a stripping current under diffusion limited conditions. With this design, the technique avoids typical complications present in voltammetry such as electrodic adsorption, homogeneous and heterogeneous kinetics, etc. and the results are robust and extremely simple to interpret. The use of the simple potential program provides safe results, but can be prohibitively long for the determination of very low concentrations. So, we have designed various strategies to reduce the deposition time: a) reduction of the preconcentration factor (3); b) a deposition program with an additional potential step in reduction diffusion limited conditions (3); c) reduction of the electrode dimension (4), d) exploitation of the contribution of complex dissociation along the diffusion layer (2,3).

AGNES has been applied to determine free Zn concentration in two coastal seawater samples taken close to Barcelona and Tarragona (Catalonia, North Eastern Spain) finding values in the range of 1-3 nM, representing around 25% of total Zn (5). This determination required the development of a new blank, called the shifted blank, which is applied in the same solution with the same potential jump, but in another region of potentials. This technique can, in the near future, be crucial in helping to elucidate the role of the free zinc(II) concentration in natural waters.

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Speciation investigation of trace metal ions using complexing resins as sensors for the free metal ion concentration in real samples

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The free metal ion concentration in a solution in contact with a ion exchange resin, in which only the free metal ion is adsorbed, is proportional to the concentration of adsorbed metal.
It can be demonstrated that $[M] = cV/K^* w$ (1,2).

c is the concentration of metal ion adsorbed in mmol/ml of the solution phase, V is the solution volume (ml), *w* is the amount of resin (g), and *K** is the partition coefficient of the metal ion between the two phases, i.e. the ratio of the metal ion concentration in the resin phase and the metal ion concentration in the solution phase.
The aim of the present communication is to show that very low free metal ion concentration can be accurately measured in this way, if the fraction of sorbed metal ion is between 0.1 and 0.9, i.e. if the conditions leading to the proper detection window are employed. Concentrations of free copper(II) and lead(II) as low as 10^{-18}-10^{-20} M can be evaluated.
Notice that the concentration of sorbed metal ion can be accurately determined after elution from the resin, by methods with low detection limits.

This is demonstrated for copper(II), aluminium(III) and lead(II), by comparing the free metal ion concentration determined by the method here proposed and that calculated from the literature complexation constants of known ligands. For all these metal ions *K** was evaluated considering the sorption equilibria which were previously characterized by the Donnan model for the ion exchange resins.

The proposed method for determining the free metal ion concentration has been proved to be helpful for the titration of unknown ligands in natural samples, for example for the simultaneous evaluation of the side reaction coefficients of aluminium(III), uranyl, copper(II) and lead(II) in a seawater sample at different acidities, and different *V/w* values. The data were treated according to the well known Ruzic-van den Berg linearization method: $\frac{[M]}{[ML]} = \frac{1}{K' c_L} + \frac{[M]}{c_L}$, modified for the acidity variation.

Very high side reaction coefficients were determined for the low metal ions concentration present in natural waters.
A further example is given by the titration of the unknown ligands of copper in an Italian wine. The presence of strong copper(II) species was demonstrated.
Potentiometry by ion selective electrodes, and other electrochemical methods, as for example ASV, have not so low detection limits in complex matrices as those here considered.

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OP 4.5

Copper speciation in different types of natural waters

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For copper, the predominant oxidation state in surface waters is Cu(II), since it has the highest affinity towards oxygenated ligands, namely inorganic as CO32- and organic with carboxylic and hydroxide groups (1,2). More than 95% of Cu(II) is transported by rivers adsorbed on particles that may be coated with organic matter. On the other hand, even in aerobic conditions, the radicals formed in the photooxidation of dissolved organic matter may reduce Cu(II) to Cu(I). This can be of special importance in sea-water and estuarine conditions, where chloride stabilizes Cu(I) (3,4). Other ligands like ammonia, some types of amines and organics with soft donor atoms such as sulphur also stabilize Cu(I) (5,6,7).

In order to investigate the influence of organic matter on copper speciation in aquatic media, namely in river, estuarine and marine waters, titration of the natural sample with copper (II), while keeping the other characteristic parameters of the solution constant, has been followed by voltammetric techniques with low detection limits. The degree of lability and type of redox reaction were analysed in order to arrive at a valid interpretation of results. It was found that labile complexes, within the timescale of the technique (about 50 milliseconds), were formed during the titration of all samples. Before titration, labile complexes were also found in fresh-waters, while inert complexes were determined in the estuarine water. In some river samples, Cu(I) species were stabilized by organic matter with soft donor atoms during the redox process at the electrode, and in estuarine waters chloride ion also contributed to their stabilization. Finally, the highest complexing strength for copper was found in the estuarine water. The sea-water did not show any complexation due to the low level of dissolved organic matter. These results point to the importance of the medium, namely, type of organic / inorganic ligands present and pH in copper speciation, which can be reflected in terms of bioavailability.

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OP 4.6

Inorganic arsenic speciation in various water samples with GF-AAS using coprecipitation

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Inorganic arsenic species, i.e. arsenite As(III) and arsenate As(V), are generally found in natural waters, while the organic arsenic species, such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are present in marine and other biological samples. Inorganic arsenics are known to be more toxic than organic ones, and As(III) is is appreciably more toxic than As(V). Inorganic arsenic is a multi-site human carcinogen matter. Arsenic in direct skin contact may cause redness and swelling. Therefore, the determination and speciation of arsenic species are very important and necessary for natural water samples such as drinking water, stream water and hot spring water samples.

In this presentation, a simple, economic, selective and sensitive method for distinguishing between As (III) and As(V) is discussed. It is based on selective coprecipitation with cerium (IV) hydroxide of As(III) in presence of an ammonia/ammonium buffer for pH 9. The coprecipitate containing As(III) is collected successively on a 0.45-µm membrane filter, the membrane loaded with the coprecipitate is dissolved with 0.5 mL of concentrated nitric acid and the solution is completed to 2 or 5 mL with ultra-pure water (resistivity > 18 MΩ cm^{-1}). Arsenic(III) in the final solutions is determined by graphite furnace atomic absorption spectrometry (GFAAS). Under the working condition, As(V) is not coprecipitated.Total inorganic arsenic is determined after the reduction of As(V) to As(III) with NaI. The concentration of As(V) is calculated by the difference of the concentrations obtained by the above determinations.

In this work, both the determination of As with GFAAS in presence of cerium (IV) and the coprecipitation of As with cerium (IV) hydroxide were optimized. The preconcentration factor was found to be 75 with quantitative recovery (≥95 %). The rsds for the replicate analysis (n=6) of 2-160 µg L^{-1} arsenik solutions were lower than 10 %. The limit of detection (3σ) for both As(III) and As(V) were 0.05 µg L^{-1}. The proposed method produced satisfactory results on the application to the direct analysis of inorganic arsenic species in drinking water, stream water and hot spring water samples. The suitability of the method for determining inorganic arsenic species in the water samples was checked by analysis of the water samples spiked with 4-10 µg L^{-1} each of As(III) and As(V). Also it was controlled with a reference method based on TXRF. The relative error was under 5 %.

OP 4.7

Flow-injection system for ultra trace analysis of mercury species by AFS

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The high mobility and toxicity of mercury, which is set free by natural and anthropogenic processes and distributed therefore ubiquitously, requires analytical monitoring of all environmental compartments. Here, especially the hydrosphere plays an important role, since mercury is enriched from water in fish and sea fruits up to 10^6 times (1). In the European water framework guideline (2) mercury is classified as one of the 33 most precarious pollutants, and thus its monitoring is strictly demanded.

Since bio transformation leads to organic mercury species with extremely higher toxicity than that of inorganic mercury, speciation analysis became more and more important in this context. Most speciation methods are based on chromatographic separation techniques coupled to varying detection methods providing differentiation of all kinds of mercury species (3). In water however only a few mercury species are solved, i.e. elementary mercury, complexes from inorganic and monomethyl mercury and dimethyl mercury (4). Therefore, it seems meaningful to develop simpler methods for affordable and reliable monitoring of certain mercury species in the hydrosphere.

The new developed flow injection system provides the successive determination of total mercury, organic Hg species, elemental mercury and inorganic mercury species in aqueous samples. Total mercury is determined without addition of reagents by separation and enrichment on an active precious metal surface in aqueous solution and can be set free as Hg^0 vapour for AFS measurements by thermal desorption. The separation of solved inorganic mercury is based on the selective reduction of Hg^{II} to Hg^0_{aq} and the selective amalgamation of Hg^0_{aq} on a smooth gold surface. This amalgamation process is carried out in aqueous solution and provides also the separation of elemental Hg from the aqueous sample. The selectivity of the gold trap was investigated in detail.

The enrichment of the organic Hg species remaining in solution takes place similar to the total mercury determination after enrichment on an active precious metal surface. The optimization of the collectors was the most important point of the new development. All individual steps of separation and enrichment are integrated into a fully automated FI system and coupled to AFS for detection. Detection limits of about 200 pg Hg l^{-1} are reached for all species when using a sample injection volume of 15 - 30 ml for a three-fold measurement.

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OP 5.1

Trace element species and its impact on human health

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Trace element species can influence toxicokinetics and toxicodynamics of elements with resultant human health effects. Speciation of trace elements is influenced by:

1. *Carriers* (elements of concern: e.g., As, Cr, Mn, Pb) - Examples: Cr(III) is unable to enter cells in contrast to Cr(VI). An anion-exchange carrier takes up Se(IV) into erythrocytes.

2. *Valence state and isotope* (elements of concern: e.g., As, Cr, Mn, Hg) - Examples: Cr(VI) is better absorbed via oral and dermal route than Cr(III). Sn(II) is better absorbed orally than Sn(IV). U(VI) is better absorbed inhalatively than U(IV). Inhaled Hg(0) is almost completely absorbed pulmonarily, inorganic Hg(I) and Hg(II) are incompletely gastrointestinally absorbed. As(III) is more toxic for the central nervous system (CNS) than As(V).

3. *Particle size* (elements of concern: e.g., Mn, Ni, Pb, Ti) - Example: Ultrafine particles of Ti dioxide produced greater inflammatory responses in rat lungs than did larger particles.

4. *Element ligands* (elements of concern: e.g., Al. As, Cr, Ga, Pb, Mn) - Examples: Astrocyte toxicity correlates with Mn-associated ligands in the order $MnCl_2 > MnSO_4 > MnPO_4$. Zn oxide and Zn chloride have potential health risks in workers. Complex halogenated salts of Pt are potent allergens in contrast to tetraammine Pt dichloride alone. The application of Gd-DTPA as contrast substance for magnetic resonance tomography (MRT) induces acute phase reaction in hemodialysis patients. Se compounds, which can generate monomethylated Se, seem to be more efficacious in cancer prevention than other Se compounds.

5. *Organic versus inorganic element species* (elements of concern: e.g., As, Pb, Hg) – Examples: Organic Hg is almost completely absorbed gastrointestinally in contrast to inorganic Hg(I) and Hg(II). Methyl Hg and Hg(0) enter the brain via blood-brain barrier than do inorganic Hg(I) and Hg(II). As(V) organic metabolites are less toxic than the inorganic forms.

6. *Biotransformation* (elements of concern: e.g., Al, As, Cr, Pb, Mn, Hg) - Examples: Hg(0) and organic Hg can be converted to inorganic Hg(II) in nearly all organs resulting in a slow redistribution of inorganic Hg(II) out of these organs. Ag precipitates and is then transformed into complexes or elemental metal that accumulates in the skin and produces a blue-gray discoloration.

Human-Biomonitoring ignores often the impact of species on bioavailability and toxicity and is then insufficiently specific for the evaluation of potential hazard and harm of environmental and/or occupational exposures. Because speciation can influence toxicokinetics and toxicodynamics, different exposure standards must be established for different element species. Further research in trace element speciation is needed to improve the measurement of potentially toxic substances and/or their metabolites and/or their human health effects. This can only be performed in cooperation between researchers in metallurgy, pharmacology, physics, toxicology, occupational health, hygiene, environmental medicine, medicine, biology, biochemistry and analytical chemistry. The outcome will clearly improve the evaluation of environmental and/or occupational exposures and the assessment of human health risks.

OP 5.2

Metallocenes as labels in bioanalysis

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Organometallic compounds are not among those substances, which obviously are well suited as labels in bioanalysis. This is due to their limited stability in the presence of air and water and their limited stability in polar solvents. However, many ferrocene and cobaltocinium compounds are surprisingly stable over the complete analytical process and are soluble in polar organic solvents or even water, respectively. The most important advantage of the metallocenes is their established chemistry. A large variety of functional groups is readily accessible, including thiol reactive maleimides or amin-reactive succinimides.

We are presenting novel metallocene-based labelling strategies for the analysis of peptides and proteins. The derivates are analyzed using liquid chromatography (LC) or capillary electrophoresis (CE) with electrospray (ESI) and inductively coupled plasma (ICP) mass spectrometry (MS) detection. While ESI-MS provides for molecular information, in particular when combined with tandem mass spectrometry experiments as neutral loss scan or precursor ion scan, ICP-MS allows the exact quantification of the metal ion. Even complex mixtures of a tryptic digest can be easily analysed. The combination of both techniques is a unique tool for protein analysis, as demonstrated in this presentation.

OP 5.3

MeCAT: Applications for absolute quantification of proteines and peptides using HPLC/ICP MS

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The ability to quantifiy properly very low amounts of proteins and peptides plays an important role in medical and biological research. This need lead to the development of several methods (1), (2) for comparative studies of a proteom within a biological system. These methods allow quantitative analyses of proteins and peptides but provide only a low dynamic range and relative high limits of detection. On the other hand the MeCAT-approach (3) developed by our group us works within an increased dynamic range and limits of detection in attomol range. The MeCAT-method uses lanthanoid chelate complexes which are covalently bound to proteins and peptides. These labeled molecules may be sequenced by MALDI and ESI MS and MS/MS and finally quantified in an absolute manner by ICP MS.

Complex mixture of MeCAT labeled Proteins require a prior separation before a quantitation by ICP MS is feasible Therefore we have established a HPLC/ICP MS setup to demonstrate the abilities of the MeCAT-Method. Using external calibration it is possible to quantifiy proteins and peptides in attomol range and at the same time over a dynamic range of 5 orders of magnitude.

The development of the MeCAT-procedure and its applications will be demonstrated on the quantification of Insulin and the quantification of mixture of proteins. Several instrumental setups are used and examined.

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OP 5.4

Use of double spike speciated isotope dilution to investigate the differences of reactivity between freeze dried and cryogenic biological standard reference materials for mercury speciation analysis

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The origins and the processes driving the inadvertent transformations of inorganic mercury (iHg) and methylmercury (MeHg) in cryogenically stored and homogenized fresh-frozen (FF) versus freeze-dried (FD) biological Standard Reference Materials (SRM) were investigated using alkaline digestion, derivatization and GC/ICP-MS analysis. Labile enriched ^{201}iHg and ^{202}MeHg isotopic standards together with their cysteine-complexed molecular analogs ($^{201}Hg(Cys)_2$ and ^{202}MeHgCys) were used in a double spike speciated isotope dilution (SID) model to study the role and influence of the complexing ligands/radicals originally associated with mercury species in these materials, on the equilibration, the reactivity and the transformation processes between endogenous and isotopic species during the main analytical steps.

The results revealed that a negligible methylation occurred in both materials, whereas a significant demethylation yield was only detected in the cryogenically stored fresh-frozen materials. Systematic investigation of the analytical steps revealed that this apparent demethylation yield, as given by the double-SID model, resulted from the possible influence of demethylating agents. However, a significant fraction of the demethylation yield was found to be potentially biased and resulted from a lack of equilibration between labile spiked iHg species and their endogenous analogs, indicating probable different complexation/lability patterns in the FF material after the extraction step. This effect was not observed in the FD material. The derivatization reaction was found to drive these non-quantitative equilibrium conditions by fractionating labile and/or weakly bound mercury species relative to strongly bound mercury complexes, leading to potentially biased iHg isotopic ratios. These differences of reactivity between FD and FF biological materials might reflect the effects of freeze-drying procedures on the stability and reactivity of mercury species and/or on the demethylating agents. Although the effect of this preparation step on organometallic species stability has been poorly described, it is known to denature proteins by inducing structural perturbations (unfolding/conformation changes). These conditions are likely to modify the binding/complexation of mercury species and potentially change the reactivity of the materials.

OP 5.5

Online coupling of 2D-HPLC and CN-PAGE for native separation of metalloproteins with subsequent detection by LA-ICP-MS

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The metabolism of trace elements and in particular their binding to proteins in living systems is of great importance in toxicological and biochemical studies. The main problem of the analysis of non-covalently bound metal-protein complexes is the low stability of the three-dimensional structure of the protein, which needs to be preserved during sample preparation. In order to conserve the integrity of as many metalloproteins as possible during separation, native separation conditions are required. Due to lower resolution of native separation techniques in comparison to denaturing methods, only multi-dimensional combination of gentle separation techniques provides both preservation of native protein conformation and good resolution.

In this work, a semi automated, three dimensional, native separation technique was developed. Two dimensional high performance liquid chromatography (2D-HPLC) was coupled to native polyacrylamide gel electrophoresis (PAGE). For the first HPLC dimension a Superdex-200 exclusion column with a separation range between 10 kDa and 600 kDa was used. Peaks, which are detected by an online coupled ultra violet/visible detector, were trapped on short (2,1mm x 30 mm) mixed bed ion exchange columns. After switching the trap column into a second flow path the next separation step was realised by passage of the sample through a mixed-bed ion-exchanger (2,1mm x 50 mm). Peaks were detected inline by an ultra-violet diode array detector. These are directly applied to a slot of native gel using a fraction collector after adapting the efflux to the conditions required for gel electrophoresis by adding an electrophoresis sample buffer using a t split. After gel electrophoresis, detection of protein-bound metals by laser ablation inductively coupled plasma mass spectrometry is possible. The state of native conformation of metalloproteins such as alkaline phosphatase (AP) and superoxide dismutase (SOD) following size exclusion, ion exchange and native PAGE separation was investigated by enzymatic assays. The applicability of this method for non-standard samples was demonstrated by determination of protein-bound metals in human serum.

OP 5.6

Analytical assay development to study metal-binding proteins in plants

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Trace elements play an important role in biochemical processes. Metabolism of the trace elements, in particular their binding to proteins in a biological system is of great importance in biochemical, toxicological and pharmacological studies. The knowledge of the specific binding mechanisms of metals within the soil, water and plants has significant implications for delineating potential bioavailability and/or release of metals. The identification of metal complexes is important to improve understanding of the tolerance and transport mechanisms in plants, which are for instance essential to phytoremediation of the heavy-metal contaminated soil as well as for risk assessment of contaminated foodstuffs.

Cadmium is one of the most widespread metals which is highly toxic to plants and animals. The concentration of Cd in foods depends on many parameters such as ability of the specie to absorb and accumulate Cd, the availability of Cd in the soil, which is itself influenced by soil characteristics (pH, cation exchange capacity). Some leafy crops like spinach are able to accumulate more than other food plants. Furthermore, non-nutritional species such as Arabidopsis halleri can hyperaccumulate Cd and can be used to clean up the contaminated soil by using the phytoremediation method.

The objective of this work was to develop (i) a suitable sample preparation method for the provision of high yields of intact metal-binding proteins; (ii) an analytical method suitable for characterization of metal containing proteins. In this research we have elaborated a new strategy for screening of cadmium containing proteins which consists of the extraction of proteins by application of an ultrasonic homogenizer, separation of the metal-binding proteins by use of native PAGE and size exclusion chromatography and their detection by ICP-MS.

OP 5.7

HPLC-ICPMS and ESI-MS to study the possible synergic effect of cadmium and selenium in induced metallothionein

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Metallothioneins (MTs) are a group of low molecular mass sulfhydryl-rich proteins with a high affinity for essential and toxic trace metals. It is also recognised that Metallothioneins play a central role in the homeostasis of the essential elements (e.g. Zn and Cu) and in the detoxification of toxic metals (e.g. Cd and Hg) in vertebrates. In fact, an increase in heavy metal concentration in cells stimulates the “de novo” synthesis of apothionein, which can then bind metal cations to produce a non-toxic form (1).

On the other hand, selenium compounds have demonstrated to produce a detoxification effect against toxic metals such as As and Hg. There is also a controversy about the effect of selenium administration on the detoxification of cadmium. While some authors reported the formation of a Se-S bond in MTs, others found that selenium compounds may catalyze the oxidation of MTs even at the reducing conditions existing in the cytosol as a whole.

Previous research in our group (2) on the simultaneous administration of Cd and Se to mussel seemed to indicate that Se did not bind to Metallothionein-Like proteins in mussel hepatopancreas (although it influenced Cd redistribution among MTs-sub-isoforms.

In this communication, the effect of selenium administration on the MTs induction by Cadmium, as a possible mechanism of detoxification, will be discussed. Two complementary separation mechanisms were coupled to UV and inductively coupled plasma mass spectrometric detection to investigate the simultaneous speciation of Cd, Se, Cu and Zn (in rat liver tissue exposed to cadmium and cadmium plus selenium). Initially, the samples were fractionated by SEC and then, the MTs fraction was further purified by RP-HPLC. This latest mechanism allows the separation of the different isoforms of metallothioneins and to study the different patterns of separation between rats exposed to cadmium or to cadmium plus selenium (2). These in vivo experiments revealed that the coadministration of Cd and Se to rats induced the synthesis of a special isoform containing Se, Cu, Zn and probably Cd which was isolated and analyzed by ESI-MS. On the light of our overall results mechanisms of selenium protection against important toxic metals will be discussed.

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OP 5.8

Measuring metallodrug-peptide/protein interaction by speciation analysis

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Determination of metal-biomolecule association in biological applications is one of the most challenging tasks of speciation analysis. Measuring these interactions on different levels of complexity (1) in experiments with selected proteins or other relevant biomolecules and (2) attempting comprehensive mapping of adducts in-vivo is of crucial importance in many research fields as e.g. bioinorganic chemistry and biomedicine. The capabilities and limitations of the complementary use of both elemental and molecular mass spectrometry in these specific applications will be presented.

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PO 1.1

Evaluation of two strains of Portuguese marine cyanobacteria in natural seawater: Biological response and chemical speciation of the culture medium

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Cyanobacteria are an important fraction of aquatic phytoplankton that occur in most open lakes, reservoirs and marine waters. They are responsible for the incidence of blooms in water bodies, which may have harmful effects on human health, and also for other organisms present in the environment (1). Even with an increasing number of reports about the aesthetic, biological, chemical and health impacts caused by these organisms in fresh water (2-4), few studies are focused on the chemical speciation of cyanobacteria cultures. Furthermore, information regarding to marine cyanobacteria and seawater is scarce.

Nowadays, major challenges in trace metal research include determining the proportion of the detectable metal that is actually bioavailable and identifying the organic chelators that help to modify free metal concentrations in natural waters (5). The toxicity of heavy metals released into the environment has increased the attention given to chemical speciation studies in which phytoplankton community has central importance due to its influence in the fate of trace metals in natural waters not only by biological surface reactions, but also by metal uptake and production of extracellular organic matter with metal binding properties (6).

In this sense, the main purpose of this study was to evaluate the biological response of two strains of cyanobacteria collected from the Portuguese coastal border when in natural seawater in vitro. The chemical speciation of the culture medium was determined in order to verify possible modifications in the culture medium as a function of the growth, by the determination of the concentrations of total dissolved trace metals ($[M]_d$), metals into the cyanobacteria cells ($[M]_{cyano}$) and released Cu-complexing ligands, by voltammetric methods.

Experiments were followed for 30 days, where control culture medium (without algae) were also carried out in parallel. Results showed, for the first time, that the *Synecocystis sp.* cyanobacterium could grow in natural seawater only enriched with N and P, while the *Oscillatoria sp.* demanded some small amounts of vitamins in the culture medium. It was also observed that *Synecocystis sp.* cyanobacterium released into the medium relatively high quantities of Fe and Zn, which concentrations reached values which can be toxic for other marine microalgae, as previously stated (7). The amount of released metals was compatible with the metal concentration in the cells (at least for Zn).

Results obtained in a laboratory scale regarding to the chemical speciation might be relevant in the establishment of possible causes of marine cyanobacterial blooms, once the availability and function of certain trace metals, combined with other environmental and physical factors, may hold the key to identifying what role these parameters play in cyanobacterial growth and ecology and would provide an understanding in how to control such blooms.

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Mending of contaminated seawaters using marine cyanobacteria. Evaluation of the trace metals removal rates and chemical speciation of the medium

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In the past years much concern over the presence of heavy metals in the aquatic environment and its cumulative toxicity and environmental impact has lead to extensive research into developing effective alternative technologies for the removal of these substances from effluents and industrial wastewater (1,2).

The conventional physical and chemical techniques currently used for the removal of heavy metals from industrial wastewater become inefficient and expensive especially when the heavy metals are present in huge volumes at relatively low concentrations.
The ability of many microorganisms to grow at high metal concentrations and sorb metals is well known and there is considerable potential for using them to treat wastewaters (3).
The use of microbial biomass for the removal of toxic metal ions from polluted effluents (biosorption) is being studied, as a potential alternative (or combined) to conventional processes and several algae have been shown to possess excellent ability to concentrate metals.

Cyanobacteria (bluegreen algae) are widespread organisms, with specific properties, such as high nutrient removal capacity and toleration to the highly variable conditions (that characterize polluted effluents) being well-suited for wastewater and remediation purposes. Cyanobacteria can be easily cultivated and hence could be valuable in metal bioremediation (4,5). Many studies have already described the use of freshwater cyanobacteria for metals removal (6-8). Nevertheless, the chemical speciation of marine cyanobacteria is still scarcely described in the literature.

In this context, the main aim of this work was to evaluate the use of different strains of marine cyanobacteria, collected from the Portuguese northern border, for the removal of selected trace metals when in natural seawater culture medium. Results were analyzed in order to establish an approach of these systems, looking forward for a potential application of these microorganisms in environmental protection, throughout the (a) selection and characterization of cyanobacteria strains to be used in metal sorption; (b) determination of the removal rates (regarding to each metal); (c) chemical speciation of the culture medium and its correlation to each studied system and (d) optimization of the parameters in metal removal.

Moreover, the use of biological processes for the treatment of metal enriched wastewaters can overcome some of the limitations of physical and chemical treatments and provide a means for cost-effective removal of metals.

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Development and validation of a method for the quantification of tributyltin at subnanogram per liter concentrations

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Tributyltin (TBT) as a compound in antifouling agents is used to protect ship hulks. This causes an increase of its concentration in sediments and surface water, especially in habours. The toxic tributyltin species decreases the number of males in fish populations due to their estrogenic activity to organisms. Organotin compounds were taken into consideration in new federal law concerning protection of soil and sewage sludge. In European directive related to the water contamination, the acceptable tributyltin concentration is very low and about 0.2 ng/l. So the determination of trace impurities concerning TBT in water requires a very sensitive technique like GC-ICP-MS, but basically common or traditionally analytical methods do not permit the measurement of this organotin species in environmental matrices.

A preliminary step has to be developed for the separation of TBT from the samples mentioned above. In addition the ultra-trace amount of TBT has to be concentrated so that the limit of detection of the measurement techniques can be efficiently lowered. Tributyltin preconcentration procedures, like solid phase extraction (SPE) using various column packings like octyl or octadecyl reversed phase materials should be established. Optimized adsorption and elution profiles for suitable recovery rates have to be developed and validated as well as a further concentration step using solid phase microextraction (SPME). For the accurate quantification of the preconcentrated TBT samples suitable derivatisation procedures were investigated using different types of alkylation reagents. The detection of these alkylated TBT species were carried out by using a GC-AED. Standard addition procedures using triple-distilled water and tributyltin standards are elucidated as well as real samples of contaminated water.

Chemical fractionation in investigation of heavy metals mobility in industrial waste polluted soils

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Proper evaluation of the effect of heavy metals on the natural environment is possible on the basis of knowledge about their forms and bindings with soil, sediment, sludge or solid waste components found. Sequential extraction could be the source of the above mentioned information, enabling identification and quantitative determination of various forms of the same chemical element. It is therefore widely used as a tool for the study of fate of metals in the environment. The loading of ecosystems with heavy metals can be due to pollution by waste materials. Landfill leachates are still one of the major sources of heavy metals discharged to the surrounding environment. The aim of this work was to identify the mobility of heavy metals in polluted soils and environmental impact of industrial waste disposal.

In experiments dewatered galvanic sludge from electroplating plant and three types of soils: sandy forest, agriculture and loamy, were used. The soil samples were taken from the top 20cm. Investigations were performed for air-dry weight of the sludge and soils samples. Each soil and galvanic sludge were mixed in the ratio of 50 to 1. The experiment was carried out on three parallel soil-sludge samples using polypropylene columns. During experiments demineralized water was added to each column. The leachates were collected to determine the quantity of leached metals. Before the start of the experiment and after 1 week, 1, 3 and 6 months, the sequential extraction of metals was carried out.

A five-step sequential fractionation scheme was used to partition the metals into exchangeable (F I), acid-soluble (F II), reducible (F III), organic matter (F IV) and residual (F V) fractions. The metals forms occurred in fractions F I - F IV can be released to ecosystems under changeable natural conditions. The metals forms occurring in the residual fraction (FV) are permanently immobilized. The sequential extraction of heavy metals from the industrial sludge and soils used different extraction buffers: (i) CH_3COONH_4 (for the exchangeable fraction); 10ml of 1M CH_3COONH_4 for 1h in room temperature; (ii) CH_3COONH_4/CH_3COOH (for the acid-soluble fraction); 20 ml of 1M CH_3COONH_4 adjusted to pH = 5 with acetic acid for 5h in room temperature; (iii) NH_2OH•HCl and CH_3COOH (for the reducible fraction); 20 ml of 0,04M NH_2OH•HCl in 25% (v/v) CH_3COOH for 5h in 95ºC; (iv) HNO_3, H_2O_2 and CH_3COONH_4 (for the organically bound fraction); 5 ml of 0,02M HNO_3 and 10 ml of 30% H_2O_2 adjusted to o pH = 2 with 65% HNO_3 for 5 h in 85ºC and 10 ml of 3,2M CH_3COONH_4 in 20% (v/v) HNO_3 for 0.5 h in room temperature; and (v) HNO_3 and H_2O_2 (for the remaining metals); 3 ml HNO_3 and 6 ml 30% H_2O_2 for 1 h in boiling temperature and 10 ml H_2O for 0.5 h in boiling temperature. The extracts were measured in a flame atomic absorption spectrophotometer (AAS).

The amounts of metals (Cu, Ni, Fe, Zn) leaching from sludge-soil samples were increasing during the studied period of time successively. The occurrence of Cu, Ni, Fe and Zn in particular fractions of sequential extraction depends on the type of investigated soil (sandy forest, agricultural or loamy).

Distribution and speciation of metals in a polluted site of Basilicata (Southern Italy)

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Speciation analysis represents the most suitable method for the study of mobility and bioavailability of the metals in soils and sediments subordinates to various anthropic pressures. To investigate the distribution and fate of some trace elements in an industrial area of the Basilicata (Tito Scalo, southern Italy), 75 samples at various depths (0, 0.5, 12, 18 e 23 meters) were collected. The samples have been characterized by powder X-ray diffraction and chemically by ICP-MS (Al, As, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, Tl, V e Zn) after sequential selective extraction and total digestion. In particular: i) exchangeable and carbonates bound metals have been extracted with ammonium acetate to pH 5; ii) fraction bounds to amorphous and poorly crystalline oxy-hydroxides has been removed by hydrochloric hydroxylamine in acetic acid at 25%; iii) metals bound to organic matter have been extracted with 30% hydrogen peroxide in HNO_3. The bulk sample was digested for complete dissolution with an acid mixture of HNO_3-HF-HCLO in a microwave system. The results obtained have shown that the main mineralogical components (quartz, feldspars, carbonates, gypsum, illite/smectite mixed layer, illite, chlorite and kaolinite) exert a minor role in controlling element distribution. Metals are mainly associated to Al-Fe-Mn oxy-hydroxides and to organic matter; some of which are also major constituents of trace minerals (Lettino et al.; this volume). Among all the elements analyzed, those that show more elevated degrees of mobility are respectively: Cd>Mn>Tl>Se>Zn in exchangeable-carbonate fraction; Mn>Cd>Co>Be>Pb in oxy-hydroxides; Se>Pb>Cd>Be>Cr in organic matter. Potential geochemical risk for human health is negligible.

PO 1.6

Mercury pollution surveys in Riga (Latvia)

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It is well known that mercury and its compounds are highly toxic to humans, ecosystems and wildlife. Although mercury is released by natural sources, additional releases from anthropogenic sources, like coal burning and use in products, have led to significant increase in environmental exposure and deposition. Mercury is widely distributed, but monitoring and control of its pollution is a relatively new activity.

We performed mercury pollution surveys at several districts of Riga (Capital of Latvia), using Zeeman atomic absorption spectrometer RA 915+. The concentration of mercury was sampled in the air above the subject of interest. The measurements have been performed mainly from the driving car. GPS was used to enable measurement results assignment to particular measurement places. Resulting setup gives a possibility to establish a digitalized pollution database for different geographic coordinates in different times.

From results of surveys one can see that background atmospheric mercury concentration in Riga generally does not exceed 5 ng/m3, but there are some places with increased mercury pollution that need particular attention and cleanup. Examples for such surveys will be shown.

There are many commonly used objects that contain mercury, for instance, mercury lamps, switches, and mercury thermometers. Disposed objects usually are thrown into trashcans, and afterwards transported to the waste dump. Measurements were performed for a number of dustcarts returning to the waste dump at Getlini. Increased mercury concentration was found in every 5^{th} or 6^{th} cart. From measurements performed in the waste dump we can conclude that mercury pollution in the household waste is a really existing problem.

The studies of mercury pollution cases draw attention to the fact that the pollution in Latvia is mainly caused by inconsiderate actions of humans.

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PO 1.7

Selective ultratrace determination of uranium isotopes in the environment

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The determination of the isotopic signature of uranium traces found in the environment is of crucial importance for the detection of undeclared nuclear waste and/or anthropogenic contaminations. The isotope of interest is ^{236}U, which is produced by neutron capture from ^{235}U. In the environment neutron fluxes are very low and thus the natural abundance of ^{236}U compared to ^{238}U is well below 10^{-10} (1). By contrast, the ^{236}U concentration in spent nuclear fuel can reach up to several percent due to the high neutron flux in nuclear reactors (2). So the uranium isotope ^{236}U is a sensitive tracer for anthropogenic uranium and can provide further information on migration and speciation behaviour of man made nuclear waste in the environment.

In a close collaboration between Mainz University and PNNL, Richland, High Resolution Ionization Mass spectrometry (HR-RIMS) is developed to precisely measure the isotopic composition in low level uranium samples. For this purpose, the initial sample is evaporated from a graphite furnace into vacuum to form a well collimated atomic beam. Neutral uranium atoms are resonantly excited by precisely tuned laser light by multi-step optical excitation steps along bound atomic states up to final ionization. Photo ions generated are guided through a quadrupole mass filter for background reduction und further enhancement of the isotopic selectivity. Finally uranium ions are quantitatively detected by a channeltron detector.

The first and most important step for highly selective ultra trace analysis of uranium using a HR-RIMS system is the identification and characterization of a suitable ionization scheme. Optical transitions between atomic levels in an appropriate excitation scheme must have narrow natural line width to provide high optical selectivity as well as sufficient transitions strength for efficiency. In that case saturation with moderate laser power is possible. Extensive laser spectroscopy on uranium intermediate levels were done by P.G. Schumann and B.A. Bushaw (3,4) and lead to a suitable excitation scheme using laser wavelengths of 415 nm, 829 nm and 722 nm.

For first tests of analytical measurements samples with known amount of about 10^{17} uranium atoms were inserted on zirconium foil and heated up to ~2500° C in a graphite furnace. A total efficiency of $3 \cdot 10^{-7}$ and isotopic ratios $^{236}U/^{238}U$ down to 10^{-8} have been demonstrated sp far. Analytical measurements were performed on a synthetic dilution series in the isotopic range of $^{236}U/^{238}U$ ratios from 10^{-3} down to 10^{-8}. These measurements confirm the linearity of the system over the full accessible range. The final characterization concerning accuracy and reproducibility will be done using certified samples from IRMM (Institute of reference material and measurements, Geel, Belgium).

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PO 1.8

Multielement speciation of mercury and tin in inland surface waters using GC-ICP-HR-MS

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Speciation is indispensable for accurately assessing physiochemical properties such as toxicity, mobility and reactivity in biogeochemical cycles of trace elements. Pollutants, such as methylmercury and organotins have generated the most interest over recent years due to their high toxicity and persistent nature. To contend with these contaminants, a series of directives police maximal concentrations for various elemental species in a number of matrices. Recent amendments to the EU Water Framework Directive (WFD) impose ppt levels of mercury species and sub-ppt levels for tributyltin compounds as annual average concentrations in inland waters.

To address the need for determination of ultratrace concentrations representative of real world samples, GC-ICP-HR-MS was evaluated as a technique which offers high resolution of species and the ultimate in sensitivity. The outcome is the ability to use a sample preparation protocol which does not require huge sample volumes in order to attain the LODs required to conform to recent legislation. Additionally, the ICP-MS allows the simultaneous determination of mercury species and organotin compounds. This presentation highlights the remarkable features of GC-ICP-HR-MS for the analysis of trace element species in a number of inland water samples from the Bremen, Germany area. Validation of the technique is performed using spiked recovery procedures and figures of merit are presented.

Iodine speciation in the marine aerosol by inductively coupled plasma isotope dilution mass spectrometry

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Iodine has a complex chemistry in aerosols in the marine boundary layer (MBL) and is involved in both ozone destruction and new aerosol particle formation processes. Much effort has been spent on analyzing iodine species in marine aerosol and on investigating the mechanism of iodine in MBL. However, many open questions remain. Sampling, identification and quantification of those iodine species have become necessary for the understanding of atmospheric iodine chemistry in MBL.

Inductively Coupled Plasma Isotope Dilution Mass Spectrometry (ICP-IDMS) was applied for iodine speciation in marine aerosol. Long-lived 129I was used for isotope dilution in iodine quantification. Marine aerosol samples including PM2.5 and size fractionated aerosol samples were collected during field campaigns at Mace Head in Ireland and on the open ocean over the North Atlantic during June-July 2006. After sampling, ultrasonic assisted water extraction was applied to collect soluble iodine species in marine aerosol. Total soluble iodine was measured with ICP-IDMS while a new online analytical technique of Gel Electrophoresis (GE) coupled with ICP-MS was also developed for iodide and iodate measurement. Furthermore, non-water soluble iodine was extracted by Tetra-methyl-ammonium Hydroxide (TMAH) extraction and analyzed by ICP-IDMS. Detection limits are both 0.1 µg/L for ICP-MS and GE-ICP-MS (expressed as iodine) iodine measurements. Water-soluble iodine was calculated by the difference of total soluble iodine and the sum of iodide and iodate. The concentrations of iodide, iodate, water-soluble organic iodine and non-water-soluble iodine will be shown in the contribution. Therein, water-soluble organic iodine and non-water-soluble iodine were the major iodine species in marine aerosol. These results indicate that more knowledge about the sources and formation pathways of the organic iodine fraction in the marine aerosol is urgently needed.

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Determination of atmospheric iodine species using a diffusion denuder and GC-MS technique

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Over the past two decades the atmospheric impact of iodine chemistry has been receiving increasing scientific attention and still is a growing research field of interest due to the potential atmospheric significance of iodine photochemistry. More recently, interest in marine atmospheric iodine chemistry has been greatly stimulated by the observation of new particle formation events in the marine boundary layer (MBL) and the suggestion that molecular iodine is likely the most important precursor for new particle formation and the dominant source of reactive iodine in the coastal MBL (1,2). Although some progress has been made, a number of uncertainties in the sources, sinks, kinetics parameters and recycling of iodine remained and the identification and quantitative analysis of some key reactive iodine species still is a challenging analytical problem.

In this study a diffusion denuder system capable to preconcentrate and quantitative analyse gaseous molecular iodine (I_2) and iodine monochloride (ICl), which are two key species in the iodine atmospheric chemistry, was developed. 1,3,5-trimethoxybenzene and α-cyclodextrins/I^- proved suitable for the collection of gaseous ICl and I_2, respectively. The respective experimental collection efficiency was 98.2% and 99.4% for I_2 and ICl at the gas flow rate 300 mL min^{-1}. The experimental efficiencies agreed well with the theoretical values for both I2 and ICl for gas flow rates from 300 to 1800 mL min^{-1}. The operation of both I_{2^-} and ICl-denuder was independent of relative humidity and storage periods (at least 2 weeks prior to and after sampling, respectively). Thus, it provides a practical, reliable and convenient protocol for the quantitative information on the sources strength in both open sea and coastal areas and the understanding of the biological and chemical mechanism of their release.

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PO 1.11

On-line speciation and determination of Cr(III) and Cr(VI) in drinking and waste water samples by reversed-phase high performance liquid chromatography coupled with atomic absorption spectrometry

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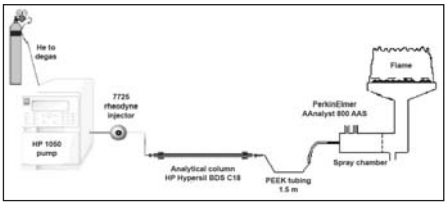
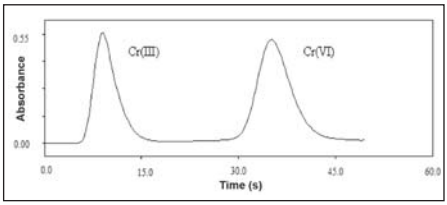
A simple, rapid, and selective on-line method for the speciation and determination of Cr(III) and Cr(VI) in aqueous solutions by ion-pairing HPLC coupled with flame atomic absorption spectrometry (FAAS) is described.



The composition of the mobile phase has been optimized for better separation. The effects of column temperature, volume of injection loop, fuel flow rate of FAAS, and nebulizer suction rate of FAAS have also been investigated.

Separation is accomplished in almost 2.5 min on a 25 cm length C_{18} column at 400C. The selectivity of the method has been established by investigating the effect of interfering elements on chromium determination. The detection limit (3σ) achieved by the method was calculated as 3.7 ng/mL for Cr(III) and 2.0 ng/mL for Cr(VI). The proposed method has been validated by analyzing certified reference material (BCR 544) and successfully applied to the analysis of drinking water and wastewater samples with a relative error below 6%.



Keywords: AAS / Chromiumspeciation / Combined techniques / HPLC / Water analysis / HPLC-FAAS

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Mercury speciation in contaminated sediments using headspace trap gas chromatography and atomic fluorescence spectrometry

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Mercury is a contaminant of great concern in the environment, particularly in water where the formation of methylmercury (most toxic form) in aquatic sediments, and its subsequent bioaccumulation in aquatic organisms, presents a major pathway for human exposure to methylmercury (MeHg). The sediment of the Deûle River in northern France is highly contaminated by many metals; this is a consequence of a smelting plant which disperses great quantities of industrial wastes, dusts and metal ores.

In this work, we present preliminary data relating to the extent of sediment mercury contamination in this site. For this purpose, Mercury contamination was investigated in surface and in core sediment. These were analyzed for total mercury, methylmercury and acid volatile sulphide (AVS) to provide more detailed information about mercury toxicity. Total mercury was measured in dry sediments (without any pre-treatment) by means of an AMA 254 solid phase Hg-Analyzer using atomic absorption spectroscopy. The organic form (methylmercury) was analysed by isothermal GC-CVAFS (Gas Chromatography Cold Vapour Atomic Fluorescence Spectrometry) after MeHg extraction from sediment into dichloromethane and reextraction into the water phase by dichloromethane evaporation. A new system "TurboMatrix HS-40 Trap" (filed with Tenax sorbent) coupled with GC and CV-AFS detector (TEKRAN) was used, this method combines a headspace introduction system with trapping process that pre-concentrates analyte before injection into the GC, which offers the possibility to develop an automated method with lower detection limit. The system HS with Trap used for analysis of methylmercury in sediments was compared with the conventional HS injection.

It was shown that combination of HS with Trap offered better performances for methylmercury determination than the HS only, regarding several aspects. Trap system offer better detection limit due to possibility of pre-concentration in several cycles, so a greater part of the analyte can be introduced into GC. This method was tested on certified reference materials sediments IAEA 405, IAEA 433 and IAEA 158 and validated for the analysis of methylmercury in high contaminated sediments. Surface sediment samples from the Deûle River were analysed for total mercury and methylmercury (MeHg) with results ranging between 2.3-78 mg kg^{-1} (dry weight) and 1.1-46 µg kg^{-1} (dry weight) respectively. The principal sediment mercury source of contamination was identified near a former smelting plant (Metaleurop) that produced lead and zinc until 2003. This high contamination suggests that some organism toxicity near this site would be expected. Sediment cores were then sampled closeness Metaleurop location. Concentrations of total mercury show an increasing trend with depth, showing that a much greater volume of contaminated sediments is present at this location.

PO 1.13

Chromium (VI) speciation through the environment

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Chromium in the environment exists as several chemical species, where oxidation states 0, +3 and +6 are the most common. The characteristics and properties of trivalent and hexavalent chromium is greatly different and chromium's speciation is strongly dependent on the chemical and physical environment. Once chromium enters the environment, its toxicity is to a large extent determined by its chemical from; Cr(VI) is toxic and carcinogenic whereas Cr(III) is considered an essential nutrient, making speciation analysis very important.

Speciation analysis requires a multi-step approach, typically including sample pre-treatment, separation and /or extraction and finally instrumental determination. It is of great importance to choose a technique that maintains the chemical speciation of the sample. Methods for chromium speciation have existed for years, however many of them are complicated and time-consuming in addition to having unsatisfactory detection limits (Kotas and Stasicka, 2000).

In this study, we make use of a simple and in-expensive ion-exchanger as means of separation of Cr(VI); where Cr(III), present as a cation, is retained on the ion-exchanger whereas Cr(VI), present as an anion, is passed to the effluent (USGS 2003). This method was developed as a simple field method for Cr(VI) speciation in natural waters. However, we have found the method suitable for Cr(VI) speciation in a wide range of environmental samples, such as precipitation, soil, sediments, sludge, biological tissue and air samples. Ion-exchange is followed by determination with HR-ICP-MS, which enables a detection limit for Cr(VI) in precipitation and natural waters of 6 ng/l. Here we present results from a large scale screening survey on Cr(VI) speciation in a wide range of environmental compartments in Sweden during spring 2007.

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Determination of Cr(VI), selected heavy metals, and elemental carbon in PM10 from a roadside sampling side in Vienna City

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Pollution from motor vehicles, in particular exhaust fumes and the wearing of disc brake pads, clutches, and tires, seems to be responsible for increased levels of heavy metals in particulate matter near roadside locations. Earlier studies have shown that, besides Sb and Fe, elevated levels of Cr also occur in particulate matter sampled near roadsides. In most cases, only total chromium, and not Cr(VI) levels are determined, which consequently provides only limited information about the toxicity and mobility.

The aim of the presented work was to assess the levels of selected heavy metals (Ba, Cd, Co, Cr, Cr(VI), Cu, Fe, Mn, Mo, Ni, Pb, and Sb) with the emphasis on Cr(VI). Furthermore, the origin of heavy metals was evaluated by calculating correlations with a marker for combustion engines, namely elemental carbon.

For this purpose a sampling strategy with specially prepared quartzfilters and appropriate storage conditions, combined with a straight forward and reliable sample preparation technique for the extraction of Cr(VI) in PM10 samples was developed. For the determination of Cr(VI), a chromatographic system with an anion-exchange column (Hamilton PRP-X100) was coupled to an ICPMS. Analysis time was less than 6 minutes and a LOD in the region of 50 pg/Nm^3 was achieved. Quality control involved spiking the extraction solutions and the use of appropriate reference materials (BCR 545, welding dust loaded on filter).

For total element analysis, the filter samples were digested with aqua regia via a microwave-assisted acid digestion. Elemental carbon was measured coulometrically after a Toluol/Isopropanol extraction step.

In addition to Sb, the elements Mn, Fe, Cu Mo and Ba showed a good correlation with the elemental carbon value. For the elements Pb, Cd, Co, Ni, as well as total Cr and Cr(VI), correlation with the elemental carbon was not observed. Values of all monitored heavy metals were in the µg/Nm^3 region. Cr(VI) concentration did not exceed values of 1 µg/Nm^3.

PO 1.15

Speciation in environmental samples: A small review

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Any detailed and realistic study of the environmental, metabolic, toxicological or other properties of chemical elements requires differentiation between its individual compounds. Some work has been done on arsenic speciation at Technological and Nuclear Institute (ITN) on Biomonitoring and Aerosol samples. Apart from arsenosugars (found in some marine organisms and algae), eight compounds are commonly assessed in environmental matrices: inorganic arsenic – arsenite [As(III)], arsenate [As(V)] – and the organoarsenic compounds monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA), trimethylarsine oxide (TMAO), arsenobetaine (AsB), arsenocholine (AsC) and tetramethylarsonium ion (TETRA). They differ significantly in toxicity, from very toxic (arsenite) to non-toxic (arsenobetaine). While the determination of total concentration in biomonitoring studies is common practice, speciation is still very scarcely used, even in small-scale environmental studies.

This work focuses on the development of analytical methodologies for arsenic speciation in lichen samples from atmospheric-monitoring surveys, since the element can be associated with natural and, especially, anthropogenic sources that usually stand out from multivariate (factor) analyses of lichen-biomonitoring data. The base material for the present study were native thalli of the foliose lichen *Parmelia sulcata* Taylor and *Parmelia caperata* (hammered shield lichen, growing on olive-tree bark), and *Platanus hybrida* either native or exposed in mainland Portugal. Aerosol samples with particles below 10 µm and between 2.5 and 10 µm were arsenic-specified as well. Following suitable field and laboratory procedures, samples were put through instrumental neutron activation analysis (INAA; k_0-variant) for total-arsenic determination. Separation and determination of arsenic compounds in aqueous extracts were carried out in a HPLC-(UV)-HG-AFS system. The extractability in water ranged from 1.9 to 32 % of the total arsenic. Arsenate was detected in all samples, in many of them as the main arsenic compound. The second most widely found compound was arsenite, followed by an unidentified cationic species. Some samples also contained an unidentified anionic compound. The overall results do not point to a direct relationship between source factors – by Monte-Carlo aided, Target-Transformation Factor Analysis (MCTTFA) – and arsenic species. Still, the potential arsenic biomethylation by lichens and its actual relevance on biomonitoring grounds are discussed by comparing lichen-speciation data with corresponding data from aerosols, collected at the same time and in the same geographical areas.

The use of isotope dilution gas chromatography - mass spectrometry for the determination of butyltin species in marine environmental samples

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Toxic organotin compounds (OTC) have been introduced into the marine environment mainly through their use as biocides in antifouling paints for vessels. Among them trisubstituted organotin compounds (e.g. tributyl tin) exhibits highest toxicity towards aquatic life at very low concentration levels (1 ng Sn L^{-1}). Therefore, reliable and sensitive analytical techniques must be used for the determination of OTC in different environmental samples.

In the present study species specific isotope dilution gas chromatography – mass spectrometry (ID-GC-MS) technique was used for the determination of butyltin species in marine environment samples. Commercially available spike solution containing ^{119}Sn enriched mono-, di- and tributyltin was used. The analytical methods applied consisted of ultrasonic extraction, followed by derivatisation with sodium tetraethyl borate, extraction into iso-octane and determination by GC-MS. For the measurement of isotope ratios (118/119 and 120/119) the most adequate molecular cluster [M-Et] was selected and simple mathematical equations for corrections of ^{13}C contribution to measurement signals were adopted from the literature. Methods were validated by the analyses of reference materials (PACS-2 harbour sediment and ERM-CE 477 mussel tissue) and by spiking uncontaminated sea water sample. Good agreement between determined and certified values was obtained.

Finally, ID GC-MS was used for the determination of butyltins in sea-water, marine sediments and mussels from the Northern part of the Adriatic Sea. Among butyltins, tributyltin was the predominant species in all samples analysed.

PO 1.17

Mercury species stability in crude oil studied by species specific isotope dilution GC-ICP-MS

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In crude oil mercury (Hg) can occur in different chemical forms associated to specific oil fractions. Hg concentrations and chemical forms vary depending on geographic locations and depth of exploitation as well as on physical and chemical conditions prevailing during oil production and transport. The most abundant Hg species found in crude oils are elemental Hg (Hg^0) and various forms of inorganic mercuric Hg (Hg^{2+}). Occasionally significant concentrations of organic Hg compounds are observed.

The main objective of this work was to study the stability of mercury species in crude oils using isotope enriched $^{200}Hg^0$ and $^{198}Hg^{2+}$. A key task was to develop reliable analytical protocols based on species specific isotope dilution GC-ICP-MS with minimal species conversions during analysis, in particular during derivatization. The suitability of different Grignard and tetra alkyl borate derivatization reagents was tested in spiked solvent- and crude oil samples. For all reagents we observed both oxidation and reduction of the added isotope tracers. For tetra alkyl borates, and in particular for sodium tetra propyl borate, these derivatization artefacts were substantially lower and more reproducible between sub samples compared to Grignard reagents.

We performed a series of experiments in which spiked crude oil samples were prepared and stored under nitrogen or air at different temperatures for up to 3 months. In all samples the added $^{200}Hg^0$ was oxidised to $^{200}Hg^{2+}$ at variable rates depending on the applied storage temperature. However, no significant reduction of the added $^{198}Hg^{2+}$ was observed with the samples and conditions used.

PO 1.18

Studies of transport and collection characteristics of gaseous mercury species in natural gases using amalgamation and isotope dilution analysis

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Mercury (Hg) is an important quality parameter in natural gases because of its adverse effects on the environment and on metal components in gas production facilities. One of the most frequently applied methods for the determination of gaseous mercury (Hg(g)) in natural gases uses double amalgamation of Hg on Au-Pt wire and detection by atomic absorption or atomic fluorescence spectrometry (AAS and AFS, respectively). This method has been evaluated in field measurements and a procedure for its application is available through the 6978-2 ISO standard method. For most applications the method works satisfactorily, but occasionally and for reasons unknown, results are found to be variable, in particular for sour gases (i.e. acidic gases, containing high concentrations of, e.g. hydrogen sulphide, carbon dioxide, or mercaptans).

We have performed studies of transport and collection characteristics of gaseous mercury species (Hg^0, $(CH_3)_2Hg$, CH_3HgCl, $HgCl_2$) in natural gases using amalgamation and isotope dilution analysis. The studies involve different Au-Pt collection tube designs, tubing materials and gaseous matrices, including air, natural and sales gas, as well as methane and sales gas to which hydrogen sulphide has been added.

The collection efficiency for amalgamation tubes was found to be both Hg species and sample matrix dependent. Hg^0, which is considered to be the dominating gaseous Hg specie in natural gas, did not show any difference in the collection efficiency for the different gases tested. Stainless steel tubing, which is prescribed by the 6978-2 ISO standard method for determination of Hg in natural gas, was found to give temperature dependent analyte losses and memory effects in the presence of high concentrations of hydrogen sulphide, typical of sour gases.

Separation and determination of mercury sulfide and mercury bound to the organic matter in river sediment

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The influence of the concentration, volume of HCl and temperature to the dissolution of black HgS and cinnabar was investigated to develop a method on separation and determination of the species of Hg bound to the sulfide and organic matter in the sediment. The dissolution of HgS in HCl increased greatly with the increasing concentration of HCl when the concentration of HCl exceeded 4mol/L, especially with the higher temperature. Furthermore, the dissolution of HgS in HCl increased with increasing of temperature. The dissolutions of black HgS and cinnabar in100mL10mol/LHCl were 10.77mg and 5.98mg at 25°C, respectively, and HgS in the sediment could be dissolved completely by HCl when the concentration of HgS in the sediment reached to be of the order of magnitude of milligram. In this study, the sequential extraction method based on that Hg bound to Cl^- is the main reason of dissolution of HgS in HCl was developed. According to the determination of samples in the sediment, it resulted that the Hg bound to organic matter was more difficult than bound to sulfide to be dissolved in HCl.

Key words: HgS; HCl; Cl^-; sequential extraction

PO 1.20

Determination of mercury species in portuguese salt marshes using capillary GC – atomic fluorescence spectrometry

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Salt marshes located near by industrialised areas can act as natural sinks for trace metals. Anthropogenic metals, associated with suspended particulate matter can be transported by tidal currents and trapped by vegetation with subsequent incorporation into sediments. Plant roots can interact with the surrounding sediment, exuding oxygen and organic compounds that influence the distribution and availability of trace metals; however, the amount of metals taken up by the plants is dependent of the metal availability in the sediment, and this is modified by the root activity. Oxygenation of upper estuarine sediments decreases rapidly with depth due to the consumption of oxygen in the oxidation process of the organic matter; however, salt marsh sediments receive an additional input of oxygen in the sub-surface layers through the well developed aerenchyma of salt marsh plants (halophytes) which transports oxygen from leaves to roots and consequently to the surrounding sediments. This supply of oxygen can alter significantly the redox status of sediments with strong repercussions on the biogeochemistry of nutrients and trace elements, namely mercury. The methylation of mercury in salt marsh sediments is poorly documented, although abundant micro-organisms and strong redox gradients between roots and surrounding sediments may favour that process. Because the sediment environment in salt marshes is exceedingly complicated it is pertinent to investigate the conversion of inorganic mercury into organomercury species. A fully automated GC coupled to atomic fluorescence spectrometer will be described for the determination of methylmercury in sediments and salt marsh plants.

Use of different simple methods for the estimation of radium concentration in a variety of environmental samples

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Radium is one of the most important radionuclides in the natural environment. Therefore precise and accurate methods of determination of radium in the different environmental objects are highly desirable (Williams, 1990). Simple and non-destructive methods can be used for high radium concentrations, while more complicated methods are needed for measurements of low concentrations, especially if the concentration of the different radium isotopes has to be measured. Usually the modern techniques like ICPMS are not applicable.

Before measurement of an environmental sample an important decision concerns the method that can be applied. For instance a low concentration of ^{226}Ra in samples can be measured by the emanation method, which is not applicable for the other radium isotopes – ^{228}Ra and ^{224}Ra. On the other hand, by means of gamma spectroscopy ^{226}Ra and ^{228}Ra isotopes can be measured in water and in soil or sediments (Lucas & Markun, 1992). Unfortunately this method is time consuming and not applicable for low concentrations of radium isotopes. A combination of solvent extraction or extraction on manganese dioxide followed by different measuring techniques like LSC or alpha spectrometry is applied for such determinations (Eikenberg, 2003, Möbius at al.1993).

Measuring procedures depend strongly on the presence of barium ions in water samples, for instance barium interferes strongly in the alpha spectrometry method (Smithson, 1990). High salinity is also a source of problems, such that it is not possible to elute radium isotopes from soils or sediments, to evaporate water samples or to mix them directly with scintillation liquid. Another source of difficulties is the quantity of available sample material.

We compare the results from different methods for samples with extremely high radium concentration in rock material (13kBq/kg); from rock and sediments with low concentrations from Antarctica (6-12 Bq/kg) and for high salinity water samples from the mineral Spas of Bad Kreuznach, Germany (15-60 Bq/L). Some of the comparisons have been done in different laboratories under cross-laboratory comparisons.

To conclude we propose some simple criteria for the choice of the adequate measurement techniques and the related chemical procedures for simple, adequate not time consuming estimation of the concentrations of radium isotopes for different applications.

PO 1.22

Chromium speciation in environmental samples using Dowex M 4195 chelating resin

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Chromium exists in Cr(III) and Cr(VI) oxidation states in aqueous solutions. The properties of these species are different (1). Trivalent chromium, the main chemical form found in foods, is essential for maintaining normal glucose metabolism (2). Cr(VI) oxidation state is detrimental to health as it may be involved in the pathogenesis of some diseases like liver, kidney, lung and gastrointestinal cancers (3).

A solid phase extraction procedure has been established for chromium speciation in natural water samples. The procedure is based on the solid phase extraction of the Cr(VI)- Dowex M 4195 chelating resin. After oxidation of Cr(III) to Cr(VI) by using H_2O_2, the presented method was applied to the determination of the total chromium. The level of Cr(III) is calculated by difference of total chromium and Cr(VI) levels. The procedure was optimized for some analytical parameters including pH, eluent type, flow rates of sample and eluent, matrix effects etc. The presented method was applied for the speciation of chromium in natural water sample with satisfactory results (recoveries > 95%, RSD's <10%). In the determinations of chromium species, flame atomic absorption spectrometer was used. The results were checked by using various reference standard materials.

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Speciation and quantification of mercury in contaminated soils of the rural area of Descoberto – Minas Gerais, Brazil

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Toxicity, bioavailability and mercury mobility in the environment depend on its oxidation as well as on its different chemical forms. Metallic mercury occurred in the Serra do Grama region, a rural area of Descoberto in December 2002. According to inhabitants` reports, “Silver Balls” appeared in this ancient gold mining area. The region of Descoberto was included in the gold exploration route that existed in the 19th century.

After mapping the region, a diagnosis of contamination and risk was made (1) and the contaminated area was isolated. Information on mercury speciation is of great importance because oxidation of metallic mercury may generate soluble species (Hg^{+2}) and, thus, make it more movable in the environment. The metilation processes, as well as the application of any intervention in order to decontaminate this area, depend on the state of the metal oxidation.

This work aimed at obtaining the first data on mercury speciation and mercury quantification in soil and sediment of this contaminated area in Descoberto, Minas Gerais. Thermo desorption technique coupled with a Atomic Absorption equipment (TDAAS) was used focusing on investigating the processes of mercury behavior in the soil of this site and Direct Analyzer of Mercury (DAM) in order to quantify the total mercury in the samples.

Concentrations of Hg from 0.0371 to 161mgkg^{-1} were found, according to the distance from the sampling site to the hotspot of contamination, already known from previous works (1). The speciation study showed the presence of both metallic and oxidized mercury in samples near the hotspot and only oxidized mercury in many samples with concentrations as high as 90 mgkg^{-1} of total mercury. It means that mercury oxidation occurred at that place, being a great part adsorbed by the soil and another small part lixiviated by the rains together with small particles of soil. This was confirmed in studies of the particulate material collected in waste tanks, placed in this area, in order to avoid the moving of this material contaminated by Hg to a stream in the surrounding area.

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PO 1.24

The determination of hexavalent chromium in industrial and environmental samples

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Industrial chromium compounds are widely used in various industrial domains including metallurgy, electronic, chromium plating, dyes and pigments manufacturing, leather and cement. As a consequence, this element is present in several manufactured products and can be released in the environment. Thus, the determination of the different forms of chromium in both industrial and environmental samples is very important as Cr(III) is considered as essential while Cr(VI) is known as a carcinogenic agent.

In order to limit the presence of chromium(VI) in the environment and protect workers of industries using chromium, the European Union sets-up several directives involving the determination of Cr(VI). For example, this compound has to be analysed in samples from car industry (directive 2000/53/EC) or in samples from waste electrical and electronic equipment (directice 2002/96/EC). Furthermore the problem of hexavalent chromium analysis is also present in industrial hygiene, particularly for the determination of Cr(VI) in workplace atmosphere. Despite the implementation of regulation concerning hexavalent chromium, only a few standardised analytical procedure are available and most of the time, there are relative to colorimetric based methods which are not applicable to such samples.

This presentation will describe the analytical tools we have developped for Cr(VI) extraction, detection and quantification for samples from industry (e.g. dust on filter, cements, corrosion preventing coatings) and environment (e.g. water, soil). Among the different analytical methods allowing Cr speciation, HPLC-ICP-MS is the technique of choice for the analysis of such samples and can be used in association with quantification by isotopic dilution for an accurate determination of Cr(VI). However, for solid samples, it is necessary to develop extraction methods for Cr(VI) without reduction into Cr(III). If alkaline extraction generally allows to obtain good results in most samples, it was shown that forcorrosion preventing coatings composed with a deposit of Cr(VI) or Cr(III) on a zinc alkaline layer, such a procedure caused reduction of Cr(VI) and only water allows extraction without degradation. When possible, results obtained by HPLC-ICP-MS after wet extraction were compared to solid speciation techniques such as XPS and XANES. For most coatings, a very good agreement was obtained between the different techniques.

Determination of phytoremediation capability of selected plant species (atriplex nitens and descurainia sophia) for lead contamination

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Phytoremediation involves the use of plants to remove toxic compounds from water and soil (Robinson et al., 1997, Singer et al., 2007). Phytoremediation is one of the environmental friendly technologies that use plants to clean up soil from heavy metal contamination. The uptake and accumulation of pollutants vary from plant to plant and also from species to species within a genus (Singh et al., 2003). Proper selection of plant species for phytoremediation plays an important role in the development of remediation methods (decontamination or stabilization), especially on low-or-medium-polluted soils (Salt et al., 1995). There are several distinct groups of plant species according to their heavy metal accumulation capability. Heavy metals enter soils through addition of sludge, composts, or fertilizers. Even with the strictest source control, domestic sewage sludge contains heavy metals because they are present in items washed down drains or toilets (Kirkham, 2006). Lead is an element of considerable environmental and toxicological interest because of its potential deleterious effects upon human health (Demirezen and Aksoy, 2004). The study was undertaken to assess the phytoremediation potential of *Atriplex nitens* and *Descurainia sophia* are known as unwanted wild plant in agricultural area growing at a heavy metal polluted site in greenhouse experiment. Lead was shown to preferentially accumulate in the *Descurainia sophia* compared to the *Atriplex nitens*. Furthermore, significant differences in the concentration levels of lead were observed in between the control and lead-exposed plants. Overall, the plants of *Atriplex nitens* and *Descurainia sophia* were found suitable for the decontamination of the metals from heavy metal contaminated sites especially, agricultural area.

In conclusion, these species could provide a new plant resource that explores the mechanism of lead hyperaccumulation, and has potential for usage in the phytoremediation of lead contaminated soil.

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PO 1.26

Water pollution.
Determination of Cd, Pb, Cu And Zn In water by GF-AAS and F-AAS

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Water pollution is any chemical, physical or biological change in the quality of water that has harmful effect on any living thing that drinks or uses or lives (in) it. The pieces of heavy metal originating from our environment, our fillings, our diet, can be so minuscule that they can easily become embedded inside some tissue in the body. The metal could be located inside the liver, for instance, the kidneys, the heart, just about anywhere in the body.

There are several classes of water pollutants. If the first are disease-causing agents, such as bacteria and viruses also the toxic metals (including the heavy metals) take an important place in what that means major water pollutants wich make water unfit to drink and may cause, in large amount, the death of acvatic life.Examples of point sources of toxic metals in waste, we remind nonferous metal procesing plants, underground mines, etc.

The parameters established for potable and surface waters are based on national regulations (recomended by E.U. Giudelines) or international standards.In this way in potable water for Copper the maximum admited level is 2.0 mg/l, for Cadmium 0,005mg/l and for Lead 0.01 mg/l, in accordance whit Council Directive 98/83/EC on the quality of water, adopted on 3 November 1998.For surface water, in accordance with Romanian law, the maximum permissible concentrations for metals are: Copper 8 µg/l, Cadmium 0.5 µg/l, Lead µg/l and Zinc 25 µg/l.

The analytic technique to determine the four components from this matrix, being a quantitative method, involves aspects like: accuracy, specificity, standard deviation, detection limit, quantification, robustness, spike recovery, reproducibility, and all these representing performance parameters of the method. To validate the proposed method, adding a certified material of reference in the water was used.

The atomic absorbtion spectrometry is a quick, reliable method to determine micro and macro elements. The analytical performance of the method exceeds the requirements of the strictest international regulations any kind of water and thus provides a rugged method for this type of samples.

PO 1.27

Determination of chromium in wastewater, drinking water and soil contaminated by tanneries, Sialkot (Pakistan)

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An investigation was carried out for the assessment of concentration of total chromium and its species: Cr (III) and Cr (IV) in soil, drinking water and effluent of tanneries of Sialkot distributed in ten clusters. 120 samples consisting of 40 samples each of topsoil, drinking water, and composite wastewater were collected from the selected tannery clusters.

Speciation analysis in water samples was followed by chelation extraction, acid digestion and atomic absorption spectrophotometric run. Soil samples were studies for Cr (III) and Cr (VI) by UV-Visible Spectrophotometer at 427 and 540 nm, respectively. The maximum concentration of total Chromium, Cr (III) and Cr (VI) in waste water, and drinking water and soil was found to be in range of Cr: 31.87 mg/l, 2.49 mg/l, and 9.12 mg/kg, Cr (III): 11.94 mg/l, 0.69, and 9.06 mg/kg, and Cr (VI):23.56 mg/l, 3.00 mg/l and 10.59 mg/kg, respectively. The concentrations of chromium contents are above the National Environmental Standards of Pakistan. It was also found that pH above 6 lead to higher concentration of Cr (VI) due to oxidizing behavior. The study recommends the treatment of wastewater before being discharged.

PO 1.28

Trace metal analysis in root crops and its fields of Islamabad (Pakistan)

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Two root crops: *Daucus carota* (Carrot) and *Allium fistulosum* (Spring onion) and its soil samples have been characterized for the physiochemical parameters (pH, electrical conductivity and moisture content) and available concentration of trace metals and their speciation through sequential extraction. Elemental toxicity was evaluated by measuring their content in leaf, stem, root and flower of these plants by using atomic absorption spectrophotometer. The results showed that elements are mostly concentrated in soil than crop parts following the sequence Cr> Zn> Ni> Pb> Cu> Cd. However, speciation depicted the concentration of Zn and Pb as Fe-Mn oxide, Cr and Cd as exchangeable, Ni as organic and Cu as carbonate bound form. The total contents measured for trace metal are found in the usual range found in these types of soils except Cd and Zn. Due to higher accumulation potential of spring onion as compared to carrot, it can be used in phytoremediation of elements in soil.

PO 2.1

Mercury speciation in shell fishes from Korea

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The most important source of human exposure to mercury and its compounds is dietary intake of methylmercury in seafood products. Methylmercury is retained in edible tissue of seafood and present in highest concentrations in predatory species such as shark, swordfish, and tuna. Mercury and methylmercury concentrations were analyzed to evaluate human exposure through the pathway of shellfish consumption in Korea. Direct mercury analyzer was applied in a gold amalgam mode for total mercury quantification. Speciation of mercury compounds was separated and measured by characterization using high performance liquid chromatography (HPLC) in association with inductively coupled plasma mass spectrometry (LC-ICP-MS). Mercury compounds were extracted from the whole body of eleven different shellfishes by adding 50ml aqueous 1% L-cysteine•HCl•H_2O and heating 120 min at 60°C in glass vials. Mercury compounds in extract were separated by a reversed phase C-18 column and aqueous 0.1% w/v L-cysteine•HCl•H_2O and 0.1% w/v L-cysteine mobile phase at room temperature. The method was validated by analyzing three certified reference materials (DORM-2, TORT-2, 1566b). The concentrations obtained by this method were in agreement with the certified reference values for total and methylmercury. Total mercury concentrations in shellfishes ranged from 0.013 mg/kg dry mass to 0.128 mg/kg dry mass. Unlike methylmercury in finfish, methylmercury in shellfish was not predominant form of mercury. Ratios of methylmercury/ total mercury determined by this method were 22-75%.

PO 2.2

Assessment of the heavy metals in the food from Romania, 2005-2006

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Exposure to heavy metals is an important problem of environmental toxicology. Most of these metals are toxic to humans, animals and plants. Man, being at the top of the food chain, is at great risk of suffering from health hazards associated with toxic metals because of bioaccumulation. The aim of this study was the evaluation of the heavy metals contents in the food from Romania area.

The study presents the results obtained in 2005- 2006 of some metals [Pb, Cd] in the food, 1869 samples: meat (469 samples), vegetables (750 samples), panification products (283 samples), juice (162 samples), diets (205 samples), in Romania. Trace elements concentrations were analyzed by atomic absorption spectrophotometry.

In all analysed samples these metals were found. Generally, a wide variation between individual samples was observed.

Meat: The mean metals levels in the meat products varied between 0.07 mg/kg Cd and 0.08 mg/kg Pb.

Vegetables: The mean metals levels in the vegetables varied between 0.02 mg/kg Cd and 0.07 mg/kg Pb.

Panification products: The results of the investigations showed a variation of heavy metals between 0.02 mg/kg Cd and 0.06 mg/kg Pb.

Diets: The mean metals levels in the diets varied between 0.03 mg/kg Cd and 0.1 mg/kg Pb.

Determinations of these chemical contaminants in food are important in environmental monitoring for the prevention, control and reduction of pollution as well as for occupational health and epidemiological studies.

PO 2.3

Methylmercury determination in fish samples by GC-ICP-MS and species-specific isotope dilution

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In the past, the accuracy and usefulness of isotope dilution mass spectrometry (IDMS) for analytical applications has been proven by many research groups. Particularly in speciation analysis where element species might not be stable throughout the analytical procedure, IDMS is a valuable tool for the investigation of species transformations. It can furthermore be used for calibration and to develop analytical methods in which transformations do not occur.
The presented work contains results of speciation analysis of mercury in fish tissue. A combination of gas chromatographic (GC) separation with inductively coupled plasma mass spectrometric (ICP-MS) detection is used for this purpose.

The sample preparation includes an alkaline digestion with tetramethylammonium hydroxide (TMAH), followed by aqueous phase derivatization with sodium tetraethylborate (STEB). Analyte derivatives are purged from the sample digest solution and trapped in n-dodecane using a helium gas-stream. Isotopically labelled spikes, enriched with ^{204}Hg and ^{201}Hg for methylmercury and inorganic mercury, respectively, were added to the samples.

A batch of samples of edible fish tissue was analyzed for their content of methylmercury ($MeHg^{+}$) and inorganic mercury (Hg^{2+}). The IDMS-results are compared with data obtained from an Automated Mercury Analyzer (AMA).

Extraction behavior and speciation of arsenic in Wakame

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TA crucial point for the investigation of arsenic speciation in marine macroalgae is the extraction step as speciation analysis usually is performed on liquid samples. Hyphenated techniques coupling chromatographic separation to elemental and molecular detectors such as ICP and ES mass spectrometry are commonly employed. The most common extractants employed for the analysis of arsenic speciation in marine algae are mixtures of methanol and water (1).

The alga under investigation, *Undaria pinnatifida*, is one of the most consumed algal species in Japan (common name wakame). Lyophilized material was already investigated by Shibata et al. (2) and the amount of liquid and water soluble arsenic extracted accounted for almost 90% of the total. In Asian food stores the alga is, however, sold in a dried state from which arsenic is very difficult to extract.

The aim of this study was to systematically investigate the influence of different extraction methodologies on the total arsenic extraction yields and the speciation of the extracted species.

Different extractants, including organic solvents and acidic and basic aqueous solutions, were tested for their effectivities in extracting arsenic. Different means of energy transfer to the sample material in solution, including microwave radiation, ultrasonication, and mechanical shaking were employed.

A procedure was developed that allows for quantitative extraction of arsenic using microwave assisted extraction (MAE) in sodium carbonate solution. The extracts obtained under several MAE conditions using water and sodium carbonate solution as extractants were compared with the aid of two AE-HPLC-ICP-MS gradient elution methods. As the flow eluting from the HPLC column was split between an ICP-MS and an ES-MS instrument, elemental and molecular information could be obtained simultaneously. After extraction with sodium carbonate solution both the number of peaks and their respective intensities in the chromatograms is greater than in extracts obtained with pure water.

Through elemental detection for arsenic, and monitoring of molecular and fragment ions in the selected ion monitoring mode of the ES-MS, two peaks could be assigned to arsenosugars.

To the authors' knowledge, this is the first time that a method is presented allowing for complete solubilization of arsenic species from wakame. Complete analysis of all species present should therefore be possible in the future.

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PO 2.5

Determination of methylmercury in fish muscle by GC-AFS

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Methylmercury is the most toxic form of mercury present in aquatic ecosystems. Ingestion of fish muscle forms an exposure pathway of mercury to humans and the determination of methylmercury in fish is therefore important. The widely used analytical procedures for methylmercury determination are based on the coupling of gas chromatography (GC) with element specific detection methods. These procedures involve a number of discrete analytical steps, comprising the extraction of the mercury species from a solid sample, preconcentration techniques, modification of analytes through derivatization, separation by chromatography and detection. Atomic fluorescence spectrometry (AFS) affords a high degree of element specifity.

In this study the techniques of headspace solid phase microextraction (SPME) and liquid – liquid extraction (LLE) for preconcentration and sampling of methylmercury in the injection port of gas chromatograph were compared. Aqueous derivatization with sodium tetrapropylborate was performed. Propylated analytes were by SPME headspace sampled with a polydimethylsiloxane-coated fused-silica fiber, by LLE extracted into isooctane phase. Both techniques are very reproducible. To detection AFS with pyrolysis of mercury species was used.

For the isolation of methylmercury ultrasonication and microwave-assisted extractions in the presence of extraction agents, such as mixture HCl and NaCl, KOH in methanol and tetramethylammonium hydroxide were tested. Extraction efficiency of the total mercury was determined using the AMA 254 mercury analyzer, extraction yields of methylmercury by GC-AFS. The precipitated matter appeared by treatment of sample for adjustment of pH to 5 and lower extraction yields caused probably by retention of methylmercury in precipitate were obtained. The pH of the sample is an important factor influencing derivatization. Two-fold microwave-assisted extraction, Folch method extraction (chloroform : methanol, 2:1), the use of three-fold extraction by acetone and enzymatic hydrolysis with protease were tested to increasing of extraction yields of methyl mercury. Certified Reference Material DORM-2 and real sample of fish muscle were analyzed.

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PO 2.6

Arsenic and its and its species in total diet supplied from Slovenian Forces

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Information on the dietary intake of trace elements by individuals or groups can be obtained by a number of direct and indirect techniques. Only direct analysis of the food consumed during a 24-h period can provide an accurate estimate of the dietary intake of trace elements (1). All other techniques used for estimation of trace elements have limitations. In Slovenia data on the arsenic content in various foods is scarce and data on its daily intake practically does not exist. The toxicity of arsenic to humans depends strongly upon the chemical form in which it is ingested. Generally organic As compounds are considered to be less toxic than inorganic arsenic, of which trivalent arsenicals are the most toxic forms. The aim of our work was to determine arsenic and its species in 20 daily military diets in year 2002 and 15 in year 2005 sampled by the double basket method. Daily meals for soldiers were prepared according to prescribed menus (2). Each composite sample of total daily diet was homogenized with a titanium blender, frozen at -24°C and then lyophilized, milled in an agate mill and stored in plastic bottles. The chemical composition of nutrients and the energy value meet the recommended military nutritional standards (2,3,4) and RDAs. For total As determination radiochemical neutron activation analysis (RNAA), with its essentially blank-free advantage and detection limit of 1 ng/g, was used. Arsenic species were determined in extracts of meals including fish using high performance liquid chromatography on anion and cation exchange columns followed by on-line UV decomposition (optional), hydride generation and atomic fluorescence spectrometry (HPLC-UV-HGAFS) (5).

The average daily dietary As intake in both years was very similar, 13.5 µg (range 7-23 µg) for non-fish based diets (12 samples) and 247 µg (range 135-545 µg) for fish-based diets (8 samples) in the year 2002. In the year 2005, the average intake was 12 µg (4-20 µg) for 9 non-fish based diets and 158 µg (42-358 µg) for 6 fish based diets. The provisional tolerable weekly intake (PTWI) of As is 15 µg inorganic As/kg body weight (6). In the present study fish intake once per week lead to remarkably higher As intake. The PTWI refers to inorganic As, whereas most As from food, mainly fish and seafood, is ingested in a less toxic organic form. Buchet et al (1996) reported that the amount of inorganic As ingested or released in the gastrointestinal tract following meals including marine organisms is, if any, negligible (7). Our results confirmed their findings. Arsenobetaine, a non-toxic arsenic compound, was detected in meals containing fish in concentrations between 0.09 and 0.30 µg/g (daily intake up to 362 µg). More toxic arsenic compounds (arsenite, arsenate, methylarsonic acid and dimethylarsinic acid) were only detected at trace levels in a few samples.

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PO 2.7

Determination of butyltin species in seafood samples by ultrasonic probe extraction and isotope dilution analysis by GC-MS

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Organotin compounds are among the most widely used organometallic chemicals. Tributyltin (TBT), mainly introduced to marine environment through leaching from antifouling paints applied on ship hulls, has received much attention since it presents adverse effects on aquatic ecosystems. Furthermore, the less toxic derivatives dibutyltin (DBT) and monobutyltin (MBT) are also of environmental concern.

In the present work a new simple methodology for the determination of TBT, DBT and MBT in seafood samples is reported. The method is based on the use of an ultrasonic probe for the extraction of butyltins from the sample and sodium tetraethylborate derivatization, both allowing the reduction of handling time to minutes. Isotope dilution analysis is used for the determination of concentration in the extract using ^{119}Sn enriched species and GC-MS. The accuracy of the proposed method was tested on spiked real samples and on two reference materials, NIES-11 and CRM-477, fish and mussel tissue respectively. The developed method was applied to different seafood samples, fish and bivalves, from the Mar Menor (Spain) obtaining concentrations in the range 8.4-1000 ng Sn g^{-1}, 4.7-550 ng Sn g^{-1} and 3.5-540 ng Sn g^{-1} for TBT, DBT and MBT respectively.

PO 3.1

A new peroxidase POX1b, biochemical characterization, suitable biosensor for hydrogen peroxide detection in biological samples

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Peroxidases-based biosensors and specially amperometric ones offer an interesting alternative for H_2O_2 detection. In fact, peroxidases (EC: 1.11.1.7) are enzymes that catalyses the oxidation of a variety of organic and inorganic compounds by hydrogen peroxide. Determination of hydrogen peroxide is of practical importance in chemical, clinical, industrial and many other fields. Peroxidases have been used in chemical, biological and clinical as well as many other fields but novel applications in biosensors design are arousing more and more interest. Horseradish peroxidase (HRP) is the most widely used enzyme for biosensors design but new sources of peroxidases are now of great interest. In a previous work, two peroxidase from garlic bulb (*Allium sativum*), designated POX_1 and POX_2 (Marzouki Mdellji S. et al 2005) were revealed by native PAGE.

In the present work, we describe the purification and some biochemical properties of a new peroxidase called POX_{1B} and use it as a biosensor to detect peroxides. POX_{1B} seems to be attractive for biosensor design since its apparent Km for H_2O_2 is much lower when immobilized (0.13 mM) than when free (0.56 mM). In addition of its storage and operational stability, POX_{1B} was found to be highly stable vs. temperature since almost 70 % of its activity is conserved at 60°C during 40 min and fool activity is retained after 40 min of incubation at 50°C and 40°C. The optimum pH was around 5 and the optimum temperature was 30°C.

Thus, gelatin was used as a matrix for enzyme immobilization on the gold electrode surface. In order to study the electro-catalytic behavior of the POX_{1B} enzyme electrode towards H_2O_2, cyclic voltammograms (CVs) were used. Amperometric measurements were carried in a three electrode cell. POX_{1B}-based electrode show great potential to be applied in hydrogen peroxide monitoring in biological samples.

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PO 3.2

Use of a microwave plasma torch coupled to electrochemical hydride generation for the optical emission spectrometric determination of As

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The use of a microwave plasma torch (MPT) with Ar as working gas coupled to an electrolysis cell was evaluated for the optical emission spectrometric (OES) determination of As. The microwave power, the plasma gas flow, the carrier gas flow, the electrolysis current, the concentration of H_2SO_4 in the catholyte and anolyte as well as the flow rate of the catholyte and the anolyte were optimized with respect to the net intensity for the As I 228.81 nm line and the precision. Under the optimized conditions a detection limit for As of 81 ng•mL^{-1} was obtained and the precision for the determination of As was found to be 2.0 % at the 3 µg•mL^{-1} level.

The interferences caused by the transition metals Cu(II), Fe(III) and Ni(II) in the range of 1 to 500 µg•mL^{-1}, the volatile hydride forming elements Sb(V), Se(VI) and Sn(IV) in the range of 1 to 100 µg•mL^{-1} as well as the depolariser NO_3^- in the range of 1 to 500 µg•mL^{-1} on the determination of As (3 µg•mL^{-1}) by EC-HG-MPT-OES were studied in detail. The presence of 100 µg•mL^{-1} Cu(II), Fe(II) and Ni(II) resulted in reduction of the signal for As by 96 %, 21 % and 57 %, respectively. The presence of 100 µg•mL^{-1} of the hydride forming elements Sb(V) and Sn(IV) caused an increase of the signal for As by 46 % and 76 %, respectively, whereas 100 µg•mL^{-1} of Se caused a suppression of 14 %. The concentration of HNO_3 was found to have no effect on the signal of As in the range investigated.

Also after an addition of the complexing agents L-cysteine and KI/ascorbic acid the interferences caused by the transition metals in the determination of As were investigated. It was found that in the presence of 100 µg•mL^{-1} of Cu and the addition of 2 % L-cysteine or KI/ascorbic acid (1:1), the relative net intensity for As was 26 % and 77 % as compared to 4 % in absence of the masking agents.

The influence of the electrolysis current, the concentration of the H_2SO_4 in the catholyte and the anolyte as well as the concentration of Cu(II) and Fe(III) in the catholyte on the amount of hydrogen produced in the cell was studied as well. The excitation temperature as measured with Ar I lines for different amounts of H_2 carried into the plasma was found to change from 4100 K without addition of H_2 to 5100 K for addition of 2 % H_2. The efficiency of the generation of AsH_3 was evaluated for different flow rates of the catholyte and the anolyte and for the electrolysis current. For this aim As was determined in the drain of the catholyte by FAAS.

The developed procedure was used for the determination of As in a coal fly ash (NIST SRM 1633a) and in two process water samples and the results of 1629 µg•mL^{-1} and 21.39 µg•mL^{-1}, respectively, were found to agree well with those attested or the values determined by ICP-OES. The procedure developed accordingly was found to be of good use when As is present as As(III), its suitability in the case of As(V) needs further investigation.

PO 3.3

Enzymatic probe sonication treatment in cadmium plants determination

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Enzymatic Probe Sonication (EPS), a new tool in analytical chemistry, combines enzymes plus probe sonication. EPS is an emerging technique that provides an effective environmentally sustainable method at low cost. It allows a sample treatment with speciation purposes in short times, with few chemical reagents (low concentration and low volume). In its first application, a quantitatively (100%) Selenium recovery from Se-enriched yeast sample, maintaining Se-chemical forms, was obtained in times as short as 15 s (1). Previous sample enzymatic treatments (enzymatic incubation or enzymatic bath sonication) needed times between 12-48 h. In addition, the speciation of other elements of relevant toxicological impact in human health (As, Hg, Cd, and Cr), are potentials targets of the EPS procedure. This new sample treatment is expected to change many sample treatments in environmental analysis, drugs or toxic metal ions control.

This work presents a new approach for the fast Cadmium determination in plants by Flame and/or furnace graphite Atomic Absorption after a small-scale solid-liquid extraction assisted by EPS. Different type of enzymes have been tried, such as cellulase, laccase and lignin peroxidase, as well as CelLytic P, a non-ionic reagent that enables extraction of proteins from fresh or frozen leaves keeping protein immuno-reactivity and biological activity. Sorghum, Brassica and Tobacco, grown in Cadmium contaminated solutions, as well as a certified reference material, NIST-SRM 1570 Spinach Leaves, were used as plant material. An attempt was made to discriminate the Cd-chemical forms using chromatographic separation (HPLC) coupled to Atomic Absorption.

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

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In natural waters, trace metals are present in a large number of different forms, such as “free”, hydrated metal ion or complexed with small inorganic ligands, small or colloidal natural organic ligands, etc. In order to understand their toxicity and bioavailability for microoganisms in environmental systems, the study of the role of different metal species is a key issue. Permeation liquid membrane (PLM) is a promising technique for trace metal dynamic speciation, because it is a simple technique, its sensitivity is high due to its preconcentration capability and the processes of metal transport through PLM are similar to those occurring in plasma membrane of biological cell. It is based on the transport of the metal ions through a lipophilic liquid membrane, by complexation with a lipophilic ligand which serves as carrier.The important parameters which influence metal speciation study with Permeation liquid membrane are the diffusion layer thickness and the diffusion coefficients of the metal ion and its complexes, the chemical association and dissociation rate constants of the metal complexes, the partition coefficient of metal ion between the test (source) solution and the membrane, the nature and concentration of the carrier in the membrane and the membrane thickness.

A general model for the steady-state flux of metal through the PLM, has been developed for solutions containing excess of ligand compared to the metal, by considering all these parameters and the associated processes. It is applicable to complexes with any degree of lability (labile, semi-labile, non-labile and inert). It leads to a number of limiting cases for fully labile, fully inert or kinetically controlled complexes. This model has been validated experimentally with complexes with varying degree of lability, in particular: Pb-NTA, Pb-TMDTA, Pb-Diglycolate, Cu-Diglycolate and Cu- N-(2-Carboxyphenyl)glycine complexes. It enables to predict the role of the different types of complexes on the bioavailability of metals for organisms.

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PO 3.5

Influence of inorganic complexes on the transport of trace metals through PLM

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The permeation (or supported) liquid membrane (PLM) has previously been used as an analytical tool to study copper, lead and cadmium speciation in natural waters, at trace concentrations. Under specific conditions (pH, concentrations), trace metals may form, with environmental inorganic ligands, neutral complexes which may diffuse passively through the hydrophobic membrane. In this study, metal (Cu, Cd, Pb) transport through the planar sheet PLM system described previously (1,2), was evaluated in the presence of major environmental inorganic ligands such as sulfate, carbonate and chloride. The first step of this work was a careful literature review of the stability constants for the metal-ligand complexes to select the appropriate conditions under which neutral complexes are formed. This revealed some data gaps amongst databases. In a second step, comparison of the metal transport with theoretical calculations, under conditions of neutral complex formation, will be presented. The role of passive transport of metal complexes will be discussed.

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PO 3.6

Vanadium speciation by chromatographic separation of V(IV) and V(V) in acidic solution followed by ICP-OES determination

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Two new methods for vanadium speciation, a cationic exchange and an anion exchange method, have been developed for application to minerals processing samples.

The cation exchange method is based on the chromatographic separation of vanadium(IV) and vanadium(V) in acidic medium followed by the determination with ICP-OES. Vanadium species exist in acidic solution (pH < 3) as VO^{2+} for vanadium(IV) and VO_2^+ for vanadium(V). The two vanadium species were chromatographically separated using a cation exchange column, Dionex IonPack CG10, and eluant 120 mmol/L H_2SO_4 at a flow rate of 1.5 mL/min. The detection limits for vanadium(IV) and vanadium(V) are 40 µg/L and 30 µg/L, respectively. Among common anions, only nitrite, NO_2^- which may act as oxidant for vanadium(IV) and reductant for vanadium(V) can cause interference. Interference from common cations has not been observed for concentration levels not exceeding 40 mg/L. The method has been successfully applied to the determination of vanadium(IV) and vanadium(V) in synthetic and minerals processing samples.

The anion exchange method used a low concentration eluent, 10 mmol/L EDTA and 14 mmol/L sodium carbonate, for the ion chromatographic separation of vanadium species at a flow rate of 1.2 mL/min. The quantitative detection limits were 0.14 mg/L for V(IV) and 0.20 mg/L for V(V) using ICP-OES detection. The method was successfully applied to the analysis of synthetic samples and mineral processing samples.

PO 3.7

Isoelectric focussing of small metal-species

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Isoelectric focussing (IEF) is a well-known separation technique for amphoteric substances, esp. proteins (IEF is the first dimension in 2D-PAGE). Besides the high resolution power, an advantage of IEF is the inherently high preconcentration factor (2-3 orders of magnitude), which makes it an extremely valuable tool for isolation/purification of analytes. Up to now, IEF has been used more or less exclusively for high molecular weight substances, such as proteins (incl. metalloproteins).

We report here first experiments on the use of semi-preparative IEF for the separation / isolation of small metal-species (MW < 1000 Da). Obviously, IEF is only effective for ligands or metal-species, which are amphoteric (i.e., which have a point of zero charge within the applied pH 3-10 gradient). Examples are shown for the ligands glutathione (GSH), 2'-deoxymugineic acid (DMA), and histidine (His), and respective metal-species of Cu(II), Pt(II), and Fe(II/III) with GSH, Cu(II)-species of His, and Fe(III)-species of DMA. Analytical methods for measuring species distributions include atomic absorption spectrometry (for Fe, Cu), spectrophotometry (thiol-groups of GSH by using Ellmann's reaction), adsorptive stripping voltammetry (for Pt), and mass spectrometry for DMA and Fe(III)-DMA.

Our results demonstrate, that IEF can be used for the separation of small metal-species, provided that (i) the respective ligand contains acidic and basic functional groups, which are influenced by metal-complexation, and (ii) the formed metal-species are sufficiently stable (i.e. no dissociation during separation). The results are discussed, in particular with respect to the use of semi-preparative IEF for isolation and purification of intact metal-species from biological material.

PO 3.8

Speciation of mercury and organomercury compounds by high performance liquid chromatography / electrospray ionization mass spectrometry

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The analytical methods used to study mercury speciation usually involve a separation step followed by element specific detection as ICP-MS. However, it does not provide structural information and can only identify the mercury species by retention times of standards in spite of increasing sensitivity. In this study, an analytical method for determination of mercury species was applied by HPLC/ESI-MS and validated by analyzing certified reference materials (DORM-2 and TORT-2). The extraction of mercury species in CRM was employed by adding 50 mL aqueous 1% L-cystein•HCl•H_2O followed by heating for 120 min at 60°C in glass vials. The extract was separated by a reversed phase of C_{18} (150 mm × 1 mm, 5 μ) with a mobile phase of 0.1% L-cysteine in H_2O (w/v) at flow rate of 50 μL/mL. In an ESI positive mode, mercury species were identified with mercury isotope distributions and pseudo-molecular ions. And their determinations were applied with a selected ion monitoring (SIM) in order to improving sensitivity and to minimizing effects of the matrix.

PO 3.9

Determination of methyl mercury in biological samples by SPME-GC-ICP-MS

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The qualitative speciation and determination of organomercury compounds at ultra trace levels are of special interest, because toxicity, bioavailability and detoxification depend mainly on the chemical form of this element. Alkylated mercury species can cross the blood-brain barrier and cause heavy intoxifications. Much is known about the mercury circle in of aquatic systems and the microbial methylation but less about the bioavailability in soil and the effect on soil feeding invertebrates. The circle of mercury in soil is of special interest because of a high methylation potential by microorganisms. Mainly sulphate reducing bacteria are responsible for the methylation of mercury (1). This omnipresents of microorganisms in the gut of soil living and feeding invertebrates is a way to alkylated mercury and accumulate higher concentrations of organomercury compounds. The development of a food chain model which shows an increase of methyl mercury concentrations by an increasing trophic level is of special interest. This could show the way of organomercury accumulation into human tissue.

The isotope specific ICP-MS coupled to gas chromatography is a highly sensitive method for speciation of volatile organometalic compounds (2). For investigation of the alkylation process, especially the methylation of mercury, different biological samples were used. As an invertebrate model organism the annelidae *Eisenia foetida* was chosen. First measurements showed a methylation of mercury and an accumulation in tissue of *Eisenia foetida*. For detailed studies, cultures of the microbial flora of the worm gut were isolated and enriched with inorganic mercury. Also the availability of mercury in soil was measured by adsorption studies.

Speciation and determination of methyl mercury in the biological samples were done by SPME-GC-ICP-MS (3, 4). Water soluble organomercury compounds were transferred into peralkylated by a derivatization with sodium tetra-(n-propyl)-borate and extracted with dichlormethane. Determination of the extracted methyl mercury was done by the isotope dilution method. Adsorption experiments were done by sequential extraction of the inorganic mercury from soil samples (5) which were measured by ICP-MS.

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Different extraction procedures comparison for the evaluation of antimony mobility in soils from an abandoned Sb-mining area

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Soil particulate phases frequently have high concentrations of metals, but the total concentrations of contaminants are not always directly related to the real risk. Nevertheless, due to the relative ease of analysis, it is a common practice in many countries, to establish environmental quality criteria based solely on the total concentration of metals in the environmental matrix. The derivation of quality objectives for the soil compartment should preferably be based on the (bio)availability and mobility of metals.

The Antimony and Arsenic distribution and mobility in soils of an abandoned mining area located in Manciano (Grosseto–Italy) were studied applying single and sequential extraction procedures. The primary objective of this work is to exploit the possibility to implement a suite of extraction tests to predict human and ecological exposures to metals, particularly Sb and As, in mine-soil. The recent increase in awareness of the toxicity of arsenic and antimony, also at very low concentrations has renewed the interest in their mobility especially in environmentally stressed region (1). Quantification of extractable forms of As and Sb in contaminated soils provides the basis for monitoring bioavailability and their mobility in environment.

The determination of total content of the toxic contaminant has showed very high concentration of As and Sb; so, to get preliminary information on the potential mobility and bioavailability of two most critical elements of the area a complex procedure of sequential extraction was performed, after an appraisal of the existing procedures described in literature (1,2,3,4). The performed procedure comprises 8 extraction steps (water soluble, easily exchangeable, specifically sorbed on mineral surfaces, bound to Mn-oxides, to organic matter, to amorphous Fe-oxides, to Crystalline Fe-oxides, and to sulphides). The higher As and Sb percentage were extracted in step 7 (more than 50% of the total content), indicating that probably their mobility depends on dissolution of Crystalline Fe-oxides.

Fractionation using this sequential extraction procedure provides comprehensive information about the potential mobility of contaminants: indeed, the more elevated is the number of steps, the more we obtain information, but this is also time-consuming. Therefore, to assess the potential metal mobility it is essential to consider also other relatively more rapid and simple procedure, furnishing adequate information about the mobility of trace elements and taking into account the kinetic aspects of the metal-sediment associations. For this reason BCR extraction procedure and kinetic test were also performed. The choice of the adequate procedure will depend on the aim of the work, the type of expected information and available resources.

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Application of mercury determination by Zeeman atomic absorption spectroscopy in forensic analysis

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Mercury has a long-drawn history of use in manufacturing of gun cartridges' essential components – primers. Such primers were produced until 60..70-ties of the last century, and though generally not in military use anymore, cartridges containing them are widely accessible in criminal weapons turnover nowadays.

Availability of highly sensitive Zeeman atomic absorption spectroscopy-based RA-915+ mercury analyzer together with the above-mentioned consideration led to the idea to try application of mercury concentration measurements in forensic investigations. Benefit of the method – its non-intrusiveness, not requiring physical or chemical action on the samples to be analyzed. Series of experiments were performed, using TT pistol, with ammunition of varying origin and manufactured in different years.

It was verified that RA-915+ sensitivity threshold 2 ng/m^3 is well-suited for detecting mercury emitted from shooting products. Prospects were verified to discover after shooting a presence of mercury in premises, on hands and clothes as well as in gun barrel and used shells, suggesting possibility of applying results of such determination in the role of arguments in criminal cases investigations. Likelihood to determine differences between shells of different origin and reproducibility of result between shells of single origin were verified. It was found that it might be possible to use measurements of mercury concentration changes for determination of shooting time, though improvements in this area are needed, and environmental conditions should be taken into account.

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PO 3.12

Selective ionophore-based optical sensor for metal ion measurement in aqueous environments

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Metals are ubiquitous. Aqueous environments can be contaminated by metal ion naturally and by human's activities. An over dose of metal ions has serious lethal effects on human health; the symptoms include bone disease, anaemia, brain damages and diabetes. The determination of heavy metals in the aquatic environment is of tremendous interest. The most common heavy metals which fulfil these criteria are iron, antimony, copper, nickel, lead, zinc, cobalt, and calcium (1).

Optical sensors have received greatest interest in the last decade for potential metal ion measurement in the aqueous environment due to its simplicity and remote applications. The application of a so-called ionophore which comprises a lipophilic ion carrier and a complexing agent capable of reversibly binding ions and transporting them across organic membranes, e.g. PVC, by carrier translocation, is an important improvement in optical sensing area (2).

This project involves the design of ion-selective optodes based on novel materials capable of selectively interacting with specific metal ions in aqueous solutions. Absorption spectrometry is the basic technique. The potential of optical sensing materials for determination of metal ions in a wastewater stream is presented. Promising results have been obtained by using chromophoric dyes: Acid Alizarin Violet N (AVN) with Fe^{2+}, Fe^{3+}, 2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol(Br-PADAP) with Cu^{2+}, Co^{2+}. 2,6-Dichloro-3,3-dimethyl-4-hydroxy-3-sulfofuchson-5,5-dicarboxylic acid (CAS) with Fe^{2+}. The complexation of a novel compound 2-145 (figure 1) with Fe^{2+} obtained new bands at pH 7 and 8 (figure 2).

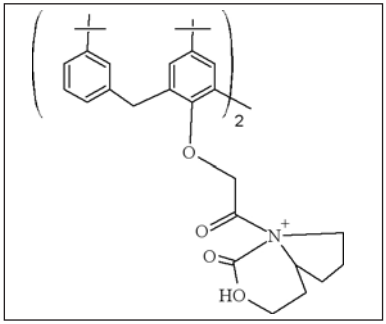

Figure 1. Novel sensing material 2-145.

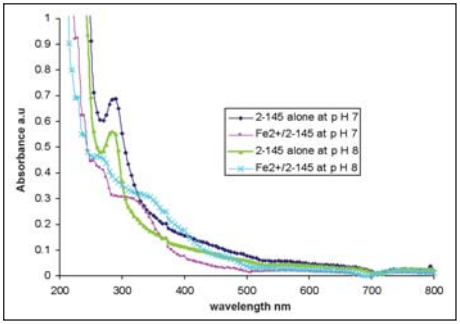

Figure 2. UV-Vis absorbance spectrum showing complexation of 2-145/Fe^{2+} at pH 7&8.

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PO 3.13

Minimization of systematic errors in ultra trace analysis of mercury species with FI-CV-AFS

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The knowledge of the biologically active species is highly important for the assessment of health risks, which might result from the distribution of toxic elements in the environment. A well-known example is the methyl mercury poisoning in the Japanese bay Minamata in the 50's of the last century (1). Due to high absorption rates (2), the bio-concentration factor (BCF) values of methylated mercury are substantially higher than that of inorganic mercury species. The monitoring of Hg species, especially of methyl mercury in the environment is therefore of great importance.

The majority of mercury speciation methods are based on chromatographic separation and previous derivatization of the species. An alternative method developed in our working group provides a non-chromatographic technique for mercury speciation analysis in natural waters based on a fully automated flow injection (FI) system coupled to a cold vapour atomic fluorescence spectrometry (CV-AFS). The species information is obtained by a set of subsequent selective chemical reactions, which finally lead to an amalgamation of elemental mercury on a gold surface. A subsequent thermal desorption followed by AFS detection (3) allows a very sensitive detection of different mercury species. However, the stability of the Hg species during analysis is one of the most important requirements in speciation analysis, generally and also in the special case of FI-CV-AFS. In this context, the stability of methyl mercury was investigated with respect to various reagents, surfaces and temperatures. The main focus was set to the stability of methyl mercury in the presence of different mild reductants and its stability and adsorption behaviour on chemically active and non active surfaces such as tubes and vessels. Furthermore the influence of elevated temperatures was examined.

The concentration of the mercury species in natural waters is usually in the ultra trace range. Therefore, special working techniques were developed to guarantee highest possible purity of chemicals and surfaces which come into contact with the analyte solutions. All investigations were performed in a clean room (class 100). Several purification methods for chemicals and cleaning procedures for containers and materials were developed.

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Head-space solid phase-microextraction of butyl- and phenyltin compounds in human urine after derivatisation with sodium tetraethylborate and subsequent determination by capillary gas chromatography with microwave-induced plasma atomic emission and mass spectrometric detectors

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Organotin compounds have been extensively monitored in various environmental compartments including water, soil, sediments and marine biota (1, 2). Reports on the monitoring of organotin compounds concentration in human have, however, remained scarce and have been the subject of intensive debate (3, 4). This may at least in part be due to the fact that analytical methods specifically targeted at measuring organotin compounds in human tissue or liquors have not been reported so far.

In order to fill this gap, a head-space solid phase-microextraction (HS-SPME) method was developed and optimized for the gas chromatographic separation and determination of the most commonly found organotin compounds in cases of human exposure. Non volatile ionic organotin compounds (butyltin- and phenyltin compounds) were in-situ derivatised to their ethylated derivatives by sodium tetraethylborate (NaBEt4) directly in urine matrix. Various parameters affecting the yield of the integrated derivatisation/extraction procedure with SPME were examined, while using tetrabutyl tin as standard. The method was optimized for direct use in the analysis of undiluted human urine samples and six compounds could be determined after a 15 min head-space equilibration time at room temperature. The selectivity of the microwave-induced plasma atomic emission detector (MIP-AED) used here as an element specific detector for chromatography allowed the interference-free detection of tin signals in all cases. GC with quadrupole mass spectrometric detection (GC/MS) was used in parallel for identification of the molecular structure of the eluted compounds in the urine samples. The performance characteristics of the developed method are given both for the determination of mixtures of these compounds, as well as for the individual determination of each compound. Detection limits at the low ng Sn/L level for all organotin compounds (on a 25 ml sample) can be achieved which makes the technique suitable for sensitive routine analysis of human urine samples, with minimum sample preparation. Finally the feasibility of the proposed method was demonstrated by applying this technique to the analysis of several human urine samples.

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Hyphenation of capillary LC with ICP-MS and on-line micro fraction collection for MALDI-TOF analysis as complementary tools for protein analysis

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Capillary and nano HPLC are the methods of choice for many separation problems due to their good compatibility with the ESI process. Recently different interface systems have been described in the literature allowing the complementary application of ICP-MS and ESI-MS under exactly the same chromatographic conditions, which is a pre-requisite for a proper matching and pre-selection of peaks due to their elemental tag.

Capillary-LC-ESI-MS is often limited especially due to matrix effects and signal suppression as a result of the composition of the used LC eluent. Furthermore MS-MS experiments have to be planed very carefully since data acquisition is often restricted to short time events, defined by the small elution windows of the separated peaks.

The combination of capillary LC with micro fraction collection on a MALDI target plate allows the “quasi” on-line coupling of LC and MALDI-TOF, which helps to overcome some of the limitations of capillary-LC with on-line ESI-MS detection.

This contribution will describe the combination of capillary LC with online ICP-MS detection for element and off line MALDI-TOF analysis for a molecule specific detection of the separated compounds. The setup developed based on a nano flow splitting device and a micro fraction collector system, which allows the direct spotting of the HPLC eluent and matrix addition on either re-usable and disposable MALDI targets. First results will be presented.

Multiplexed probing of cytochromes P450 using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)

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Cytochromes P450 (CYP) are among the most important enzymes involved in the metabolism of xenobiotics. CYP profiles as co-determinants of adverse effects vary substantially and are subject to xenobiotic-dependent modulations. Hence, profiling methods for CYPs, associated receptors and involved signaling compounds are an important tool for toxicological research. ICP-MS has often been used in recent times for the element specific detection of proteins. On one hand, it can be utilized directly to detect the phosphorus in proteins through which phosphorylation status of proteins could be monitored. On the other hand, proteins of interest could be labeled and indirectly observed concurrently (1).

Considering the different hyphenation techniques for ICP-MS, laser ablation is found to be the most suitable one for SDS-PAGE separated proteins. Laser ablation of blot membranes after PAGE separation gives the required quantitative information of proteins with high accuracy as it has lesser volumes, minimal contamination and is easy to handle compared to gels. Based on these aspects a new laser ablation cell has been designed and adopted (2, 3). For concurrent determination of proteins in a sample, downstream applications such as immuno-blotting could be useful provided that specific antibodies are available. We use two differentially labeled monoclonal antibodies for detection of CYP1A1 and CYP2E1 by LA-ICP-MS. These CYPs are present in rat liver microsomes after 3-methylcholanthrene and isonicotinic acid hydrazide treatment. In addition expression of the same CYPs from the duodenum of minipigs was analyzed and compared. The CYP1A1 antibody was labeled with Europium via a covalently coupled chelator (DOTA = 1,4,7,10-tetraazacyclo-dodecane-tetraacetic acid) and the CYP2E1 antibody was iodinated. Suitability of the labeled antibodies for detection of blotted microsomal CYPs by LA-ICP-MS and the detection sensitivity will be demonstrated. Simultaneous application of the two differentially labeled antibodies to determine several expressed CYPs will be demonstrated.

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PO 3.17

Identification of arsenobetaine degradation products by means of HPLC - parallel ICPMS- and ESIMS-detection

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With respect to the assessment of toxicity of new arsenic species in the environment and especially in foodstuffs their quantification and identification is vitally. LC-ESIMS can also be applied for elemental and molecular detection using different fragmentor voltages but needs more time for subsequent runs. Using both techniques separately (LC – ICPMS, LC – ESIMS) showed the big potential in speciation analysis. Come together of ICPMS for elemental information (quantification) and ESIMS for molecular information (identification) after splitting of the eluent behind the chromatographic separation could be a suitable way for analysis of arsenic species for which standards are unavailable.

The simultaneous parallel ESI- and ICP-MS was applied to identify possible metabolites during the interaction of arsenobetaine with natural zeolites. Zeolites are well known sorbents to remove heavy metals and metalloids from waste and drinking water. Previous investigations on sorption of arsenic species on natural zeolites showed adsorption of arsenic compounds besides transformation and metabolisation to more toxic species. Under this aspect the interaction of non-toxic arseno-organicals with zeolites broad applied as sorbens in water treatment and newly as additives in foodstuffs and animal feed was of special interest. Arsenobetaine mainly produced by freshwater and marine organisms is known to be a candidate of low-toxicity. To estimate the possible toxicological risk originating from arsenobetaine in contact with natural and synthetic zeolites, small particles (<1mm diameter) of a natural occurring zeolite (Mexico) of clinoptilolite type was mixed with an AsB solution (5mg As L^{-1}) and stored for several days at room temperature.

After a contact time of 50 days the degradation of AsB proceeded with different yields in the case of the natural Mexican zeolites but to the same products. In contrast no additional components could be detected in the control samples (AsB only, and AsB with synthetic zeolite).

By overlaying and comparison with peaks monitored by ESIMS the degradation products DMA (m/z 139) and DMAA (m/z 181) could be identified clearly on the basis of retention times and their molecular mass $[M+H]^+$. Two additional remarkable peaks with retention times between 400 and 430 s were also detected, but could be identified neither by means of their molar mass nor of the appropriate fragments. Another arsenic species could not be identified so clearly with $[M+H]^+$ m/z 165, because two isomeric forms can exist (dimethylarsinoylpropane (DMAP) or dimethyl (3-hydroxypropyl) arsine (DMHPA). Indeed the single quadrupole MS was not sufficient for identification of isomeric compounds.

Evaluation of enzymatic-assisted extraction protocols for the analysis of total arsenic and arsenic species from individual leaves of terrestrial plants by means of IC/ICPMS and ICPAES

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Different enzymatic-assisted extraction protocols were evaluated regarding their suitability for the extraction of total arsenic and arsenic species from individual leaves of Tropaeolum majus plants contaminated by phenylarsenic compounds. Regarding the diminution of the sample size for total arsenic determination a high biological variability between different leaves and stalk segments was ascertained. To avoid a false estimation of extraction yields their calculation was based on the arsenic concentrations remaining in the extraction residues.

Using a two-step extraction comprising incubation of ground leaf material with cellulase followed by digestion with trypsin 7 ± 3% higher arsenic concentrations were extracted compared to non-enzymatic water extraction. For non-ground intact leaves an elevation of the extraction yield by means of enzymes was not achieved although a large fraction amounting to 50 up to 70% of the total foliar arsenic was dissolved from intact leaves in water within four hours without any further sample pretreatment. These results indicate a high mobility and solubility of phenylarsonic acid dominating in leaves independently on the application of amino- or nitro- and hydroxy-substituted phenylarsenic species. A cleavage of the functional groups from the phenyl ring was deduced from the arsenic species analysis using ion exchange chromatography coupled to inductively coupled plasma mass spectrometry. Some differences between the arsenic species distributions in aqueous and methanolic extracts and in enzymatic extracts were observed.

Due to the high water extractability of the arsenic species the improvement of the extraction yield using enzymatic-assisted extraction remained relatively low but the enzyme-extracted fraction of foliar arsenic can be assumed to be bound to biological macromolecules such as carbohydrates or proteins.

PO 3.19

Speciation of alkylphenols after labeling with ferrocene

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Phenols form a group of polar components of many fossil materials like crude oils. They are toxic and fairly reactive, thereby potentially causing stability problems of technical products. They are also studied in petroleum exploration and measured in many environmental situations. Despite these wide-ranging applications, there is still need for rapid, accurate and sensitive analytical methods for phenols that will allow the different species to be individually quantified.

We have found that ferrocenecarboxylic acid chloride is an excellent derivatization regent for phenols (and, to a lesser extent, alcohols) (1, 2). The reaction is rapid and sample work-up following the derivatization is limited to filtration through a short column. The determination of phenols is done using gas chromatography with either an atomic emission detector (AED) or a mass selective detector (MSD). Gas chromatography has a high separation power which is fundamental to resolve the complex mixture of isomers typically found in crude oils and products thereof. The AED relies on the iron atom which can be measured with an excellent sensitivity (2.5 fmol iron compound injected) and with a selectivity versus carbon of over 3.5 million. With the MSD, the typical fragmentation pattern of the esters can be used to obtain a phenol-selective gas chromatogram (and, in addition, an alcohol-selective chromatogram using a different ion). The prominent molecular ion can be used for speciation of differently alkylated phenols.

Examples of the analysis of alkylphenols in heating oil before and after desulfurization will be presented. A speciation of the differently alkylated phenols is desirable to distinguish between sterically hindered phenols, which contribute to the lubricity of the fuel, and unhindered phenols, which can be problematic when they form polymeric oxidation products. Changes of phenol patterns during desulfurization of fuels can give important information about the reaction mechanism on the catalyst surface. We have analyzed heating fuel, desulfurized with two different methods, at different stages of the desulfurization process. The results show that the desulfurization methods lower phenol concentrations to a different extent and vary in their effect on phenol patterns.

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Application of nuclear instrumentation methods for characterization of diffusion membranes

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Membranes are widely used in chemistry separation, in hydro-geological studies, in biotechnology processes and innovative energetic. In this study the effective diffusion through polymer and ceramic membrane is characterize with the radon gas. Advantage of the use of radon is that it gives response not only for the process itself but it can also be used for the parameterization of the membranes. Radon is an inert gas that decays quickly, producing particles (isotopes) of lead and polonium.

By using standard gamma spectroscopy we obtain reliable result for the quantity of captured in the membrane particles, as they are radioactive. From other side the diffusion process is measured with quasi-alpha spectroscopy device. We obtain in real time signal of the diffusion not only of the gas radon but of its decays products too. The results are surprising. Normally under the radioactivity decay low should to be expected equilibrium between radon and its products. Due to capture of the decay products in the pore of the membrane we obtain this equilibrium between the 18th and the 23rd hour after start of the experiment.

All these methods allow us to parameterize how the different factors, related with the membrane as active surface, thickness, structure influence the process of diffusion.
Further measurements with scanning microscope and autoradiography to detail the points of interaction between these particles and membrane will be performed.

Determination of the surface coverage of DNA conjugated gold nanoparticles by simultaneous determination of gold and phosphorus

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Functionalized gold nanoparticles play a major role in bioanalytical applications, due to their unique optical and chemical properties. Gold nanoparticles conjugated with oligonucleotides, for instance, have been employed as probes in a variety of DNA detection methods due to their sequence-specific hybridization properties. Their potential in bioanalytical applications results from an increased selectivity that can be used to discriminate perfectly complementary DNA or RNA targets from those with single-base mismatches.

For the quantitative analysis of the aggregation-linked assays, it is essential to know the exact number of oligonucleotides attached to one nanoparticle. In this study it could be demonstrated, that inductively coupled plasma spectrometry (ICP) is well suited to determine phosphorus and gold concentrations in functionalized gold nanoparticles in a simultaneous fashion, thereby rendering possible the determination of oligonucleotide coverage. During the analysis, phosphorus in gold conjugated oligonucleotides and unbound phosphate in solution have been differentiated. Methodology and results are presented.

PO 3.22

Automated multiple determination of Hg-species in marine biota by GC-CVAFS[1] after TMAH[2] digestion and solvent stripping

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Cold Vapour AFS can be run with high sensitivity, is easily to be handled and – last but not least – is one of the definitely low budget techniques. These characteristics make it be the detection method of choice in the special field of gas chromatographic speciation analysis of mercury in marine biota.

In order to complete this method's advantages, we did some efforts to find out a convenient serial digestion procedure and to make it's GC sampling mode fit for liquid injection. Complete speciation can be achieved in one unique procedure in which methylmercury, inorganic and elementary Hg as well as other volatile species are quantified. Varying some experimental details enables total mercury to be determined via one GC-Signal.

Our analytical procedures include following steps:

Speciation of methylmercury and Hg^{2+}:
Digestion by TMAH, alkylation by STEB[3] (alt.: STPB[4]), solvent-thermo stripping by N_2 into n-decane, automatic liquid injection, GC on a 5 µm wide-bore capillary column, AFS-detection as Hg0 via pyrolysis in a 920°C quartz cell

Total mercury:
Generally cold vopour AAS is the method of choice for the determination of total mercury due to its quick and simple performance. Nevertheless GC-VCAFS can also be applied for this purpose via ethylation of Hg^{2+}.

For both ethylated species limits of detection and quantification can be established in the sub-ng/g range. Thus the mercury burden of nearly every type of marine biota can be speciated quantitatively. Thus the up to now used measuring of merely total mercury in fish can be replaced or completed, providing more detailed analytical and toxicological information.

However: Due to the time consuming (because necessarily moderate) digestion conditions the total procedure of our speciation analysis takes all in all 18 hours time. This is much too long, when fresh fish has immediately to be checked prior to or while being marketed. In cases like these CV-AAS (available without any sample pre-treatment if necessary) should further on be preferred.

[1]GC-CVAFS: cold vapour atomic fluorescence spectrometry
[2]TMAH: tetra methyl ammonium hydroxide
[3]STEB: sodium tetra ethyl borate
[4]STPB: sodium tetra n-propyl borate

PO 3.23

SC-FAST: Fully automated low pressure system for performing trace elemental speciation by ICP-MS

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The ESI SC-FAST rapid sample analysis system utilizes a 6 port injection valve to reduce sample uptake and wash-out times in standard ICPOES and ICPMS analyses. With the addition of a low pressure column, a simple but effective means of performing fully automated separation of elemental species of both Chromium and Arsenic is possible.

The Chromium species are separated using a low pressure ion exchange column, the Cr(VI) species passes straight through the column while the Cr(III) species undertakes selective adsorption/desorption on the column.

The Arsenic species separation is also performed using a low pressure ion exchange column, but by step gradient elution of the different As species. The loading of the different eluents onto the column is controlled fully by the SC-FAST software.

A peristaltic pump is utilized to pass the samples/eluents through the column, providing a low cost solution to speciation analysis.

Separation, calibration and recovery data will be shown for Cr(III) & Cr(VI) in various waters, along with As(III), As(V), AsBet & MMA data in both waters and biological fluids.

Investigation of diffusive gradients in thin films technique applicability for mercury speciation measurements

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The environmental mobility and toxicological effects of mercury are strongly dependent on the chemical species present. Free metal ion and labile inorganic complexes play an essential role in the methylation of Hg since they are the Hg species available for methylation. Because concentrations of mercury in water samples are very low, accurate analysis is still major problem. Other problems are associated with collection and handling of samples. It is well known that chemical forms of metals in sample can change because of preservation and storage of sample. All these problems can be overcomes by application of in situ techniques. Recently a new technique for in situ sampling solutes known as the diffusive gradients in thin films technique (DGT) has been developed. In our laboratory we have modified this technique for measuring of mercury in natural waters and we have investigated its applicability for mercury speciation measurements. The DGT technique measures only those metal species that are able to pass through the diffusive gel and that are likely to be captured by resin immobilized in thin film gel. Use of different resins with different capture efficiencies enable to estimate fractions of mercury bonded in different complexes. In presented work we summarized the results from DGT mercury measurements in river water and in fresh and saline sediment pore waters. The resins used during the work were Chelex-100 with iminodiacetic acid functional groups and Spheron Thiol with Duolite GT73 containing thiol functional groups bonded on different matrix.

PO 3.25

Potential for the speciation of Al in human serum using convective interaction media (CIM) fast monolithic chromatography with ICP-MS and cap nano LC ESI-MS detection

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An analytical procedure using anion-exchange separation support based on convective-interaction media (CIM) was developed for the speciation of Al in human serum. The separation of proteins was performed on a weak anion-exchange CIM diethylamine (DEAE) fast-monolithic disk. To prevent co-elution of low molecular mass (LMM) Al species with high molecular mass (HMM) Al compounds on CIM disk serum proteins were first separated from LMM-Al species by the use of size exclusion chromatography (SEC). For this purpose 1 mL of serum was injected onto SEC (Superdex 75 HR 10/30) column. Isocratic elution using 0.05 M TRIS-HCl + 0.03 M $NaHCO_3$ was applied and separation of proteins was followed by UV detection at 278 nm. It was experimentally proven that proteins were eluted in 5.5 mL peak that was collected into a polyethylene cup. A 0.1 mL of the sample aliquot was then injected onto the CIM DEAE disk. The separation of serum proteins was obtained in 10 min by applying linear gradient elution from 100% buffer A (0.05 M TRIS-HCl + 0.03 M $NaHCO_3$) to 100% buffer B (A + 1 M NH_4Cl) and followed by UV detection at 278 nm. Separated Al species were detected on-line by inductively coupled plasma mass spectrometry (ICP-MS). Well-resolved protein peaks were obtained. It was experimentally proven that 90 ± 3% of Al in spiked serum of renal patient was eluted under the transferrin peak. Transferrin was identified on the basis of the retention volume and also by the capillary nano liquid chromatography electrospray ionization mass spectrometry (cap nano LC ESI-MS).

The proposed speciation procedure removes LMM-Al species and enables reliable determination of the concentration and composition of Al bound to proteins by CIM DEAE-ICP-MS and cap nano LC ESI-MS when the concentration of Al in serum is higher than 5 ng mL^{-1}. In comparison to chromatographic columns CIM disks enable faster separation and simpler manipulation during cleaning procedure and coupling to ICP-MS.

PO 3.26

Comparison of external calibration and isotope dilution in the determination of tributyltin (TBT) in seafood by GC-ICP-MS

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Organic tin species such as tributyltin (TBT), are man-made persistent pollutants that are omnipresent in the global environment. Their use as pesticides, marine anti fouling agents and as stabilisers in PVC has led to their detection in sediments, open waters, drinking waters, seafood, human blood and liver. Their well described effect on genitals on dogwhelk and detection in other biological matrices such as blue mussels, has led to an increasing demand for high throughput analysis of samples without compromising on the trueness and precision of the analytical results.

Isotope dilution mass spectrometry (IDMS) has been deemed a superior method in many ways to other methods of quantification due to its many advantages. Among these advantages is the potential ability to achieve accurate results with better precision, and that the analyte recovery does not have to be quantitative providing isotopic equilibration has occurred. However, the IDMS method also suffers from some drawbacks since in order to achieve the optimum results one has to know the concentration of the analyte in question in the sample prior to analysis. A much simpler method of quantification is the external calibration (EC) without internal standard, where the samples are analysed together with known concentration of the analyte in standard solutions. Nevertheless, the EC also suffers from some serious drawbacks. Due to the lack of internal standard the accuracy of the analysis may be seriously affected by instrument drift and incomplete recovery of the analyte.

This study describes a direct comparison between EC and ID methods of quantification of TBT using gas chromatography inductively coupled plasma mass spectrometry (GC-ICP-MS). The certified reference materials BCR-CRM 477 (mussel tissue) and NIES-CRM 11 (fish tissue) were extracted along with relevant seafood samples. Both acetic acid/methanol and tetramethylammonium hydroxide (TMAH) extractions were used for the EC and ID determination. The results from this comparison will be discussed together with implications for large volume routine analysis of seafood samples, which may have large variations in their content of TBT.

PO 3.27

Focused-microwave assisted extractions for speciation of Hg, Sn, Cr and As from environmental samples

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Sample preparation is the key point in speciation analysis in order to preserve the species integrity during extraction. The need of fast and reliable procedures can be attended with the use of microwaves as heating source to speed up the process. Speciaton analysis performed by focused microwave assisted extractions was developed during the past years yielding good performance and achieving short extraction times. Nowadays this technology has experimented a quantitative rise in terms of quality, producing products with better control in the parameters controlling the extraction (microwave power, temperature and pressure).

In this work we present different methodologies to perform the analysis of a variety of relevant species for the environment: Monomethylmercury, Tributyltin, Dibutyltin, Monobutyltin, Arsenobetaine, Chromium (VI)…

After species extraction, two main approaches are presented in this work. Extraction, alkylation and liquid-liquid extraction in case of Hg and Sn where volatile species can be formed and direct speciation of the extract for Cr and As. Where it was possible species specific isotope dilution analysis was performed as primary method for the analyte quantification. In all the cases certified reference materials were analysed to validate the method proposed.

PO 3.28

Spray chamber optimization for the coupling of CE to ICP-MS

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In analytical chemistry, speciation analysis has steadily gained more importance in recent years. This is mainly due to the fact that relevant information about a sample, e.g. toxicity, degree of contamination etc., can only be obtained if individual species of one element are determined. The coupling of inductively coupled plasma mass spectrometry (ICP-MS) with separation systems such as liquid chromatography (LC), gas chromatography (GC) or capillary electrophoresis (CE) allows both the selective and sensitive analysis of element species.

In 1995, Olesik et al. (1) reported on the first interface for the coupling of CE to ICP-MS. However, the applicability of this interface was only limited as the pneumatic nebulizer dealt with a laminar flow. In order to overcome this limitation, another nebulizer has been developed by Prange et al. in 1998 (2). Currently, two nebulizer systems are commercially available: a concentric nebulizer from Cetac, and a parallel-path nebulizer from Brugener Mira.

In this work, the parallel-path nebulizer has been used in combination with different spray-chamber designs, all of which are home-made. We used an micro cyclone spray chamber and axially spray chambers optionally with an tangential gas flow. The spray chambers have been investigated and optimized regarding nebulizer-gas pressures, flow rates and sheath-flow influences. For the characterization of each spray chamber, we show the Mg^+, Pb^+, In^+, $[CeO]^+$, and Ce^{2+} signal traces. Furthermore, the separation of cobaltocinium salts by CE coupled to ICP-MS with an optimized interface design has been investigated.

To compare the results obtained with the home-made spray chambers, a commercially available spray chamber has been used in combination with the concentric nebulizer from Cetac.

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Single cell quantification of platinum in chinese hamster ovary cells treated with *cis*-platinum (*cis*-diamminechloroplatinum(II)) using LA-ICP-MS: A new tool for the comparative analysis of single CHO-9 cells by LA-ICP-MS and optical microscopy

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In recent time more and more applications for analytical atomic spectrometry in the broad field of biological research are discovered. The methods for analysis of trace elements bound to biomolecules are advancing fast. About thirty years ago Rosenberg discovered the inhibition of cell division by *cis*-platinum (*cis*-diamminechloroplatinum(II)). Nowadays *cis*-platinum is used in chemotherapy to treat various kinds of tumours including those of testis, neck, lung, cervix and ovary. It is generally accepted that cis-platinum induced cytotoxicity results mainly from adduct formation of $[Pt(H_2O)_2(NH_3)_2]^{2+}$ with the genomic DNA, triggering apoptosis, cell-cycle arrest and the induction of chromosomal aberrations (CA).
Here we describe the combined application of light microscopy and laser ablation inductively coupled plasma mass specrometry (LA-ICP-MS) to individual Chinese hamster ovary (CHO) cells. This approach provides both information about chromosomal aberrations (optical microscopy of metaphase cells) and also information about the uptake of platinum on a single cell level.

Chinese hamster ovary cells (CHO) are exposed over night (20 h) to cis-platinum at concentrations from 1,6 to 20 µM. Cells are trypsinised, hypothonically shocked and fixed in methanol/glacial acetic acid (3:1) before they are spread on microscopy slides. Nuclear staining is performed by GIEMSA, which allows light microscopy of interphase nuclei and metaphase chromosomes at a high resolution level. Chromosomal abberations induced by cis-platinum are counted and categorized. After transferring the slide into the ablation chamber the cells are retrieved by the imaging facility. All LA-ICP-MS parameters were tuned for maximum sensitivity on phosphorus as well as on platinum. Analysis of phosphorus and platinum content of these single cells is performed afterwards by ablating the complete cell by one single laser pulse using the laser ablation system coupled to an inductively coupled plasma mass spectrometer (LA-ICP-MS). Quantification of these small amounts was performed by comparison of integrals of the ICP-MS signal resulting from the ablation of superficially applied known amounts of a matrix matched standard. The amounts of platinum uptake depending on the concentration in the growth medium can be found in the fg (10^{-15} g) range per cell while chromosomal aberrations of nearly all types can be observed. First results were fundamentals and indicated that this new approach is a capable method for the simultaneous analysis of single cells on an optical and elemental level. Future work will aim on optimization of the detection and on more complex biological questions.

Determination of germanium sesquioxide (Ge-132) by gas chromatography with microwave-induced plasma atomic emission after derivatisation

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Although germanium-containing dietary supplements have been on the market for more than 30 years now, they only recently became popular as remedies for certain diseases. Organo-germanium compounds are considered to promote health and cure diseases. They are moreover described as antioxidants and immunostimulatory medicine, e.g. inhibiting the progress of cancer and AIDS and destroying cancer cells (1,2). In most cases, germanium is used in the form of beta-carboxyethylgermanium sesquioxide ($(GeCH_2CH_2COOH)_2O_3$ or "Ge-132"), and as spirogermanium, germanium-lactate-citrate or in other unspecified forms. For humans, germanium is not essential and in general the toxicity of the mentioned organo-germanium compounds is low. Acute and chronic toxic effects of inorganic germanium dioxide have been demonstrated (3). It is obvious that especially inorganic germanium has a higher potential of negative effects. Therefore, a widespread analytical product control is indispensable.

Analytical methods for the analysis of Ge-132 presented so far have the shortcoming that they either do not speciate the individual form of Ge present in the sample, or speciation is done, however, without being able to determine the actual form of organogermanium compound present, since this is, e.g., only determined by the difference in the hydride generation signal (4), or by ICP-MS (5).

We present here a completely new approach to the determination of Ge-132, based on derivatisation by chloroformate reagents and subsequent analysis by gas chromatography with atomic emission detection (GC-AED) or mass spectrometry (GC-MS). This approach provides the convenience of aqueous phase derivatisation of the analyte by ethlychloroformate and element- or compound specific detection of the formed derivative which is extracted into a suitable organic solvent. The structure of the derivative could be tentatively identified by GC/MS (and complementary LC/MS measurements) as $Cl_3GeCH_2CH_2COOCH_2CH_3$ which is formed through hydrolysis of the Germanium sesquioxide and its subsequent reaction with ethyl chloroformate. The proposed reaction was useful for quantitative analysis down to low ng amounts of organogermanium compound (as Ge), by far sufficient for the monitoring of organogermanium levels in nutritional supplements.

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PO 3.31

New approach of transmission electron microscopy coupled with EDXS for heavy metal speciation in environmental/ biological materials

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The elemental speciation is largely required to understand the toxicology of particular heavy metal. Scientists always looking for unique non destructive technique, which can give the information of the particular metal within the cell and it's binding with different cellular material. The electron microscopy technique including scanning electron microscopy, transmission electron microscopy (TEM) coupled with EDXS has been used to evaluate heavy metal species from biological tissue which are environmentally exposed. Heavy metals present in tissues are arsenic, copper, cadmium chromium and mercury etc. These heavy metals bind to different protein and enzymes in our body. Heavy metal alteration and weathering feature can damage the cells.

The use of electron microscopic analysis is an important in present study which helps to evaluate heavy metal and mineral species in biological tissue and attempt to detect the localization of heavy metal in biological tissue. Several researchers used hyphenated techniques in combination with various types of chromatography with inductively coupled plasma mass spectrometry which is not capable to give the information of metal in which form due its destructive sample processing as well as its chemical property. In present status environmental speciation with the hyphenated techniques is required. There is a growing need for more comprehensive approaches including the direct analysis samples especially for the elemental speciation of heterogeneous materials with microscopic methods of analysis. Methods for solid-state speciation analysis with several types of beam methods of analysis needed to be reviewed.

The processing of the material should be very important step for estimation of heavy metals. The staining of biological materials should be avoided in case of heavy metals speciation. This technique is also utilized for speciation of different type of environmental materials. In case of biological material or biological tissues speciation can be done of different element within the cell. The speciation of different heavy metal is very informative for those who studying toxicology. We can localize bioavailability of different trace metal in tissues with the help of this technique.

PO 3.32

Analysis of arsenic compounds by CE/ESI-ToF-MS and CE/ICP-MS

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In recent years, arsenic speciation has gained more and more importance. It is well known that the toxicity of arsenic compounds strongly depends on the chemical form, in which they are present in a sample. Organo-arsenic compounds are less toxic than inorganic compounds are. However, organo-arsenicals are the species predominantly present in marine organisms. Arsenic is abundant in seafood at concentration as high as several hundred microgram per gram. Therefore, in countries where food from marine sources is an important part of the daily diet, it is essential to know the concentrations of individual arsenic species in order to estimate possible negative health effects. Thus, a selective and sensitive analytical method for the identification and quantification of arsenic compounds in food samples is required.

The coupling of highly efficient separation techniques such as capillary electrophoresis (CE) to sensitive and selective mass spectrometric detection allows the qualitative and quantitative analysis of arsenic compounds. Analytes can be distinguished according to their different migration times. Main advantages of CE over liquid chromatography (LC) are high separation efficiency, short separation times, small sample amounts and the possibility to effectively separate charged analytes. The use of inductively coupled plasma mass spectrometry (ICP-MS) allows obtaining elemental information about the sample and is ideally suited to quantify elements even in very low concentration ranges. The additional coupling of electrospray ionization time-of-flight mass spectrometry (ESI-ToF-MS) yields molecular information allowing to elucidate the structure of arsenic compounds.

In this work, we describe an analytical method for the determination of four arsenic species: Arsenobetaine, dimethyl arsenic acid (DMA), As(III) and As(V) are separated by capillary electrophoresis. Subsequently, the identification and quantification of arsenics are obtained by coupling ESI-ToF-MS and ICP-MS for detection. For the validation of the method, a variety of real samples has been analyzed and results have been compared to those obtained by established methods.

PO 3.33

Analysis of gadolinium-based MRI contrasting agents by CE/ESI-ToF-MS and HILIC/ESI-MS

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Gadolinium-based contrasting agents (CAs) have been widely used to enhance the contrast of images in magnetic resonance imaging (MRI) procedures since almost thirty years. Among these, the most well-known compounds are Gd-DTPA, Gd-DTPA-BMA and Gd-DO3A. Due to their excellent magnetic properties Gd-based CAs nowadays are administered in about 20 million MRI procedures per year. However, recent publications have reported that serious complications occurred after the administration of Gd-based CAs in an increasing number of cases. Especially patients with a renal failure have shown symptoms of poisoning or have suffered from a severe hardening of the skin and the inner organs (nephrogenic systemic fibrosis, NSF).

It is assumed that the renal failure and the resulting higher dwell time of the CAs in the body, cause transmetallation reactions of Gd by other metals like Fe, Cu or Zn, thus leading to an imbalance of the blood electrolyte levels. To investigate the correlation of possible transmetallations and observed diseases, it is necesary to simultaneously determine Gd-chelate, the free ligand, and possible transmetallation complexes.

In this study, the development of a method for the analysis of Gd-based CAs by hyphenated techniques such as CE/ESI-ToF-MS and HILIC/ESI-MS is presented. Gd-DTPA was separated from free DTPA and transmetallation products like Fe-DTPA, Cu-DTPA and Zn-DTPA by capillary electrophoresis (CE) and hydrophilic interaction chromatography (HILIC), respectively. Electrospray ionization mass spectrometry provided the necessary selectivity and sensitivity with limits of quantification (LOQ) down to $1 \cdot 10^{-5}$ M. The methods developed have been applied to a Magnevist® infusion solution (Bayer Schering Pharma, Leverkusen) containing Gd-DTPA as the pharmaceutically active compound.

Speciation data from voltammetric methods: Ni in xylem sap – a case study

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It is well known that metal speciation in natural systems is determined by the mixture of metals and complexing agents present, as well as, by the pH, ionic strength and major ion composition. Changes in one or more of these parameters may affect the physiochemical distribution of a metal among all of its possible forms as well as the rates of formation or dissociation of the different species. This may cause perturbations that may have significant ecological consequences if the rate of reestablishment of chemical equilibrium is slow compared to rates of competing processes. So in complex natural systems in order to understand the process that govern metal distribution and uptake fluxes both thermodynamic and kinetic effects must be taken into account (1).

Voltammetric techniques are powerful not only to measure ultratrace analyte concentrations but also to determine the distribution of the various species of a given element and physical parameters of the medium such as stability and kinetic complexation constants (2). In this work we report a study of Ni complexation in xylem sap of Q.ilex as well as the kinetics and mechanism of Ni chelation using square wave and cyclic voltammetry at a hanging mercury drop electrode. Holm oak (*Quercus ilex*) is the dominant tree growing on the serpentine soils of northeast Portugal, characterized by elevated soil concentrations of Ni and Mg, combined with low Ca concentrations. Apparently *Q. ilex* does not suffer from excessive concentrations of Ni in the soil.

The results obtained showed that the complexes with the carboxylic acids dominate nickel speciation. Mixed complexes, Ni + oxalic acid + citric acid, are also formed in the range of concentrations found in the xylem. As to the kinetics of the association/dissociation reactions, in the operational time scale used, simple as well as mixed Ni complexes with the carboxylic acids behave as dynamic, while those with the amino acids are inert (3). From the voltammetric data apparent pseudo first order rate constants of complex formation and dissociation were determined. To test the validity of the methodology, formation rate constants of the ML complexes with citric and oxalic acids in the absence of the alkaline earth cations were evaluated that compare with the anticipated values from Eigen mechanism. The measurements show the pronounced influence of Mg(II) on the kinetics of Ni(II) complexation in a media where this alkaline earth ion is present in excess to the main organic ligands of the xylem sap. The net result is a retardation of complexation reactions that can be more important for intrinsically slow-reacting metals as is the case of nickel (4).

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Optimizing conditions for labelling proteins with 2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid

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We are using laser ablation ICP-MS to detect hetero-elements in proteins and antibodies after separation by use of sodium dodecyl sulphate polyacrylamide gel electrophoresis and blotting onto a membrane (1).

In this study proteins which don't contain hetero-elements are labelled with different lanthanides using 2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid as chelating compound and a linker as well. The reactive isothiocyanato group makes covalent attachments to surface and terminal amino-groups. The effect on the rate of protein conjugation by changing parameters like pH, temperature and time is investigated and results will be presented. The optimized labelling conditions will be applied for labelling of specific antibodies used for detection of cytochromes P 450, a group of iron containing proteins expressed during chemical stress of test animals.

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High Rresolution ICP-MS trace element analysis of proteins fractions obtained from size exclusion high pressure chromatography of human cerebrospinal fluid

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The central nervous system is surrounded by cerebrospinal fluid (CSF). CSF provides a biological matrix for trace element analysis reflecting the living brain. A method to study the protein binding pattern of trace elements in human CSF is described. Proteins in CSF-samples were separated according to size by Size Exclusion Chromatography combined with High Pressure Liquid Chromatography (SEC-HPLC). A size exclusion column, Superdex 75 (10/300GL, Tricorn), and HPLC system (Hewlett Packard, series 1050) with quaternary pump, degasser, manual injector (100 µl loop) and a UV-detector (254 nm) was used for the separation of proteins in the CSF samples. Pump speed was set at 0.750 ml/min, and fractions were collected for 40 min. The column was calibrated to separate proteins in the range 6-70 kDa. Fractions were then analysed off-line using High Resolution Induction Coupled Mass Spectrometry (HR-ICP-MS) to determine trace element composition. We were able to measure more than 10 elements of clinical chemistry interest in the CSF fractions. Results will be presented for Cd, Mn, Fe, Pb, Cu and Zn. HR-ICP-MS has technically very low detection limits and the technique is particularly useful for multi-element determinations in small samples of biological material with low concentrations of trace elements. In is vital that the eluents of the HPLC is tolerated by the plasma and the inlet system of the mass spectrometer. SE-HPLC uses non-denaturating mobile phase at physiological pH which stabilizes the original metalloprotein complexes and is well tolerated by the HR-ICP-MS system. No sample preconcentration was needed for this method.

Analysis of cisplatin adducts to oligonucleotides of enzymatically digested DNA using HPLC-ESI-iontrap-MS

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Cisplatin is one of the leading metal based drugs which is widely used in treatment of cancer, especially effective against genitourinary tumors (1).

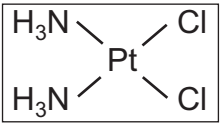

Figure 1. Structure of Cisplatin

By aquation of the Cisplatin inside the cell, the two chloroligands, as shown in figure 1, are replaced by the nucleophilic N7 positions of the purine bases of the DNA to afford primarily 1,2- or 1,3-intrastrand and a lower number of interstrand cross-links (2).

In our study, calf thymus DNA was incubated with Cisplatin for about 72 hrs resulting in the formation of various platinated-oligonucleotides adducts, which were then digested by a combination of benzonase, unspecific nuclease, alkaline phosphatase, to remove the terminal phosphate group, for 24 hrs and nuclease S1, the endonuclease specific for single stranded polynucleotides, for only a short time, 30 min at 37°C.

Separation and structure identification of platinated trinucleosides diphosphates either intrastrand or interstrand cross linked adducts was carried out using micro HPLC/ ESI-MS.
In addition to the trimers, platinated tetranucleosides tri- and diphosphates adducts were also separated chromatographically and their structures were characterised as doubly charged.

Structure elucidation for each adduct was performed through the interpretation of the fragmentation data obtained from MS-MS and MS^n experiments.

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Interactions of peptides and proteins with arsenic species and metal ions: Investigations by means of electrospray ionization mass spectrometry (ESIMS)

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The various arsenic species identified in living organisms show a different toxicity. However, the influence of arsenic species on proteins and especially the mechanism of interaction are not well understood. Inorganic arsenic species arsenite and arsenate as well as heavy metal ions are assumed to induce peptides with a high cysteine content like metalothioneins and phytochelatines as a metabolic response preventing cell toxicity (1). For the elucidation of biochemical effects of arsenic at the molecular level in dependence on the chemical species, extraction, enrichment, and detection methods are to be developed that preserve the native form of such complexes. Electrospray ionization mass spectrometry is suited for the investigation of intact protein-ligand-complexes (2).

In the presented mass spectrometric binding studies for arsenic species and the divalent metal cations Cu^{2+} and Zn^{2+} with thiol-reactive biomolecules characterized by growing molecular masses, the amino acid cysteine (121 g mol^{-1}), the tripeptide glutathione (307 g mol^{-1}), the nonapeptide isotocin (966 g mol^{-1}), and the protein thioredoxin (11688 g mol^{-1}) were used. In addition, lysozyme (14.3 kDa) was chosen as a model protein containing four structure-determining disulfide linkages. Trivalent and pentavalent arsenic was incubated with amino acid, peptide and protein solutions both as organic compound (phenylarsine oxide, phenylarsonic acid, dimethylarsinic acid) and as inorganic compound (arsenite and arsenate). After incubation of phenylarsine oxide with cysteine, glutathione, isotocin, and thioredoxin the mass spectra showed a covalent binding between arsenic and sulfur, which was stable at acidic pH values as well as in neutral solution (3). Furthermore, the influence of the solvent composition particularly with regard to the water concentration on the intensity of the mass signals for the arsenic-containing reaction products became evident owing to the release of water as second product (4). Interestingly, under the same conditions no interactions of inorganic arsenite or arsenate could be measured. In presence of added Cu^{2+} ions all mass signals caused by a covalent reaction of phenylarsine oxide and glutathione disappeared. In these mass spectra only the oxidized form of glutathione was found because of the catalytic activity of Cu(II). Regarding the model protein lysozyme, no interactions with arsenic could be detected, whereas definite Cu- and Zn-lysozyme complexes with a stoichiometry of 1:1 and 2:1 for Zn^{2+} ions and Cu^{2+} ions, respectively, were observed. For these metal complexes a pronounced pH dependency was ascertained. Another kind of non-covalent complexes was found for solutions containing pentavalent organic arsenic species and glutathione. From the presented results a different mechanism of interaction of metal cations and the metalloid arsenic with thiol-functions in amino acids and peptides as well as with sulfur-containing proteins can be concluded. This behaviour proved by ESI-MS investigation poses the question if the detoxification of metal ions and arsenic, which is often mentioned in the literature, can be really unified concerning the role of thiol reactivity. Regarding the arsenic compounds tested a strongly dependence of stable thiol interactions on the chemical species not only in terms of arsenic`s redox-state but also in the presence of a phenyl ring can be deduced.

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Exhalation of trimethylbismuth after oral application of a bismuth salt – evidence for biomethylation of metals in the human body

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Biomethylation and hydride formation of metals and metalloids is a ubiquitous environmental process that can lead to the formation of chemical species with significantly higher mobility and increased toxicity. While much is known about the interaction of metal¬(loid)s with microorganisms in environmental settings, little information has been gathered on respective processes inside the human body as yet. Here we study the excretion and biotransformation of bismuth following ingestion of a bismuth-containing pharmaceutical.

Following satisfactory medical screening, 19 male volunteers were given two tablets of bismuth subcitrate. The mass of bismuth ingested by each volunteer was 215 mg. Following ingestion, the concentrations of bismuth and its hydrated and methylated species were monitored in breath, blood, urine and faeces samples over a 56-hour period via ICP-MS or low temperature-gas chromatography- inductively coupled plasma-mass spectrometry LT-GC-ICP-MS.

Most of the inorganic bismuth ingested was excreted via faeces, only 1 % was exported via urine. Faecal bismuth concentrations ranged between 59-2356 mg/kg (wet weight), in comparison bismuth concentrations of urine were between 22-12795 µg/l. Maximum bismuth concentrations in blood were lower in the range of 1.3-158.8 µg/l. Trimethylbismuth was detected via LT-GC-ICP-MS in the breath of all volunteers typically two hours after ingestion. The sigmoid-type profiles observed during the observation period show absolute maxima after eight hours for most of the volunteers, with the concentration maxima being within a concentration range of 0.78 to 458 ng/m^3. Trimethylbismuth was also detected in blood up to 2.47 pg/g, but was not found in all cases.

The determination of Trimethylbismuth in breath and blood after oral application of an inorganic bismuth salt, which is predominant exported via faeces, is an evidence for the biomethylation of metals by the instestinal microflora. The toxicological relevance of these findings must be observed in further studies.

PO 4.7

Detection of Ni-species and Co-species in single living plant cells with high lateral resolution

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Nutrients in air, water and soil as well as ions and metals constitute the basic living conditions of all organisms. Environmental interferences like industrial pollution, mining, traffic, etc. affect both, the concentration (e.g. by accumulation) and the chemical state of individual heavy metals. Plants may take up these metals, some of them being essential for their metabolism. Adversely, they may act toxic as well, such affecting the metabolism of an organism. In the course of the food chain, thus accumulated heavy metals acquire in animals and finally in humans, with the consequence of similar toxic effects. The toxicity of heavy metals directly correlates with their chemical state (e.g. oxidation state, state of binding), so that further interpretations necessitate the analytical discrimination between the individual species.

Standard analytical techniques in element speciation with high sensitivity and high specificity focus on bulk materials, like cell cultures in biological research. A prerequisite for the interpretation of the underlying reaction mechanisms, however, is information on exchange reactions between the individual living cells – even individual cell compartments – and the surrounding fluid phase.

Analyzing heavy metal species in single cells needs high lateral resolution techniques combined with extreme sensitivity for individual species. The required standards may provide optical techniques, sensitive for fluorescence and autofluorescence like confocal laser scanning microscopy (CLSM) or scanning near-field optical microscopy (SNOM). Detection of fluorescent heavy metal species with CLSM resp. SNOM may yield a lateral resolution down to 100 nm respectively 50 nm. Fluorescent dyes with high specificity to individual heavy metal species may provide basic information, on the distribution of heavy metals in a single cell.

Standard fluorescent dyes are available for labelling heavy metals or even individual metal species, e.g. Newport Green DCF for Ni(II) and Co(II). Its fluorescence is increases with Ni(II)/Co(II)-binding and may be detected CLSM in fluorescence mode. In a pilot experiment plant cells were incubated with Ni-species and $CoCl_2$ (100 µM for 120 h). At 24 h intervals, one specimen was incubated with the fluorescent dye Newport Green DCF and then visualized by CLSM. Soluble Ni-species – $NiCl_2$ and $NiSO_4$ – tended to be taken up by the cell and were stored mainly in vacuoles and in the cytoplasm. After less than 24 h fractions of the initial Ni(II) additionally could be localized in the nucleus. Additionally, the insoluble species (Ni_2S_3) was acquired in the cytoplasm and the vacuole as well. In contrast to the soluble Ni-species the species in the nucleus fluoresces with 48 h retardation but much weaker. Under these conditions the CLSM technique can discriminate between soluble and insoluble Ni-species. In nutrient only solutions (without metal-species) for other 120 h the Ni(II) is depleted (decreasing fluorescence in the cytoplasm and the vacuole) from the cells after 120 h of incubation. Accordingly, in cells incubated with Ni_2S_3 the fluorescence in the nuclei almost completely is quenched. Similar to Ni_2S_3, Co(II) ions are stored in the cytoplasm and in the vacuole. Extended heavy metal exposure, with respect to the nucleus results in more intensive fluorescence.

PO 4.8

A new enzyme-assay for PLA_2 activity in jellyfish venom based on phosphorus detection using HPLC-CC-ICP-MS

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Although their function in the marine ecosystem is not yet fully understood, jellyfish seem to become important key species in the environment due to the limited number of natural enemies, global warming effects and environmental pollution especially with micro nutrients. Mass occurrences in some regions indicate a strong impact on the local food webs with negative effects on all trophic levels due to their continuous ingestion of different prey species. Fishing tentacles and mesenteric tentacles, which are equipped with specialized cells harbouring venom containing capsules (nematocysts) are used for prey capture and self defence.

Up to now only little is known about the chemical composition of the jellyfish toxin cocktail, the chemical structures and the biochemical effects. To understand their role in the environment it is essential to gather more information about the mechanisms and the compounds especially, which are mainly responsible for the toxic effects. The complexity of the venom cocktail requires an effect orientated fractionation strategy in order to distinguish toxicologically active and inactive fractions and to reduce the complexity of the sample.

In several reptile and insect venoms phospholipase A_2 (PLA_2) is known as a major compound. The phospholipase A_2 family is an enzyme class, which catalyze the hydrolysis of phospholipids at the *sn*-2 position to release the corresponding fatty acid and lysophospholipid. PLA_2-activity of the jellyfish venom may be responsible for some typical inflammation reactions like pain or oedema and toxic effects such as myo- or neurotoxicity in the affected organism. Additionally PLA_2 can destabilize the cell membranes and alter the cell permeability. The responses caused by a fatty acid activated cascade reaction whose products are responsible for the inflammation reactions.

This contribution will present first results on the development of an assay for the investigation of the venom of *Lion's Mane jellyfish* with respect to PLA_2-activity. The main goal is to separate and simultaneously determine the phosphorus containing enzyme substrate and the reaction products caused by the PLA_2-activity by using high performance liquid chromatography (HPLC) online hyphenated to collision cell inductively coupled plasma mass spectrometry (CC-ICP-MS). Changes of the enzyme substrate and the product quantity directly indicate an PLA_2-activity of the sample fraction under investigation. Sonicated vesicles consisting of phospholipids are applied as enzyme substrate. Reversed phase liquid chromatography (RP-HPLC) has been used to separate the lipid and the lysophospholipid. Molecule ions formed and interfered with the sensitive phosphorus specific detection, are minimized by using helium as cell gas. The established assay should allow effective indication of PLA_2-active compounds in different fractions of nematocyst extracts to guide their final identification during a multidimensional separation process.

Speciation of selenium in animal tissues using high performance liquid chromatography with on-line detection by inductively coupled plasma mass spectrometry

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Selenium is recognized as an essential trace element in the living organisms and as an important component of the antioxidant enzymes that protect cells against free radicals that are produced during the normal oxygen metabolism. The range between essentially level and toxicity level of selenium is very narrow. Selenium intake below the daily recommended amount can lead to numerous diseases caused by the deficiency of selenium, but also with the consumption of selenium in high doses its toxic effect is observed. Selenium has an important protective role against various forms of cancer and helps in prevention of heavy metal toxic effects. The influence of selenium on the metabolism depends not only on its total amount but mainly on the chemical form, which are uptake and metabolized. Therefore the identification and quantification of its species is crucial in a view of understanding of its metabolism and its importance in biology, toxicology, clinical chemistry and nutrition.

In this study inductively coupled plasma mass spectrometry (ICP-MS) coupled with anion-exchange high performance liquid chromatography (AE-HPLC) was used for the determination and speciation of selenium in animal tissues. A set of animal samples origin from the sheep exposed to the feed enriched with selenium and conjugated linoleic acid were used. The extraction of selenium species were performed with various media: water, SDS, driselase, lipase, protease as well as the mixture of lipase and protease. The use of ultrasounds or mechanical stirring assisted extraction was also compared. By using anion-exchange chromatography the presence of Se(IV), Se(VI) Selenomethionine and methylo-Se-cysteine together with few unknown selenocompounds were detected in extracts.

Speciation analysis of selenoproteins from human serum by affinity chromatography hyphenated to inductively coupled plasma-mass spectrometry

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Selenium is an essential element for life but is toxic at levels little above those required for health. The diverse biological effects of selenium cannot be explained by the chemical characteristics of the element itself or the few inorganic selenium compounds that living organisms are commonly exposed to. Inorganic selenium is assimilated and incorporated into a variety of organic compounds with distinct pharmacodynamic or toxic potentials, such as selenoproteins. In the last few years it has been revealed that understanding the reactions and functions of such selenium (protein) species is the key to understanding its mechanism of essentiality and toxicity, hence their speciation analysis is important.

In this study a new analytical approach based on affinity (liquid) chromatography (AF-HPLC) hyphenated to inductively coupled plasma-(quadrupole) mass spectrometry (ICP-QMS) was developed for speciation analysis of selenoprotein P (SelP), gluthatione peroxidase (GPx) and seleno-albumin (SeAlb) from human serum. Despite the analytical advantages of the on-line coupling of AF-HPLC, the determination of GPx by ICP-QMS is seriously hampered by Cl and Br, which produce serious interferences on ^{77}Se ($^{37}Cl^{40}Ar$) and ^{82}Se ($^{81}Br^{1}H$). Off-line anion exchange-solid phase extraction (AE-SPE) was carried out before the chromatographic separation in order to remove the spectral interfering elements. Efficient AF-HPLC separation of GPx, SelP and SeAlb free of interferences was obtained within a total chromatographic run time below 15 min. The repeatability in terms of relative standard deviation (RSD, %, n=10) was 7% for GPx and SelP and 3.5% for SeAlb. On-line external calibration by injecting inorganic selenium into the chromatographic system was used for the quantification of selenoproteins. Method detection limits obtained using this approach were 0.1 ng g^{-1} for GPx, 1.5 ng g^{-1} for SelP and 2.0 ng g^{-1} for SeAlb. The method accuracy in terms of total selenium was assessed by the analysis of a human serum certified reference material (BCR-639) with a certified content of total selenium. Total selenium obtained for BCR-639 with the speciation approach (GPx+SelP+SeAlb) agreed with the certified value. In addition, the method accuracy for the analysis of both control serum and BCR-639 was checked using direct ICP-QMS and ICP-sector field-MS (ICP-SFMS). In both cases quantitative recovery of selenium from BCR-639 and agreement with total selenium by speciation (GPx+SelP+SeAlb) for the control serum was obtained.

The potential of gel electrophoresis coupled to ICP-MS and MALDI-MS for the determination and characterisation of cisplatin DNA adducts

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The use of elemental mass spectrometry was successfully applied to different questions in biological and medical research in the last few years. Especially, the online coupling of powerful separation methods like liquid chromatography (LC) or capillary electrophoresis (CE) to inductively coupled plasma-mass spectrometry (ICP-MS) has gained great importance to the determination of biopolymers and their interaction with metals. Different methods were developed for the separation and detection of metalloproteins or DNA metal adducts in various samples. However, the most popular and powerful separation method for biopolymers, gel electrophoresis (GE), was not coupled online to ICP-MS for such studies at this time. Several approaches have been developed on the basis of laser ablation (1), but this technique is quite laborious and time-consuming, so easier approaches are highly desirable.

In this paper we describe the technical realisation of an online coupling of GE to ICP-MS (2) for the direct determination of cisplatin DNA adducts. For these studies, different 8-mer oligonucleotides are incubated with cisplatin under physiological conditions and the reaction products are quantitatively monitored via ^{31}P and ^{195}Pt detection. The high potential of ICP-MS in biochemical application is shown here for the determination of the kinetic reaction constants for the monitored processes. Complementary studies with MALDI-MS were conducted on fractions sampled from the GE system and confirmed the expected structures.

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PO 4.12

Cadmium detoxification in *Physcomitrella patens*

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The moss *Physcomitrella patens* tolerates cadmium concentrations up to 10 µM. While only small amounts of the metal were found to be adsorbed to the cell wall most of the cadmium is taken up into the cells. Our investigations have shown a strong increase of the intracellular thiol pool (cysteine, γ-glutamylcysteine and glutathione) in response to cadmium stress while no phytochelatins were detectable. After 3 days of exposure to 10 µM Cd^{2+} the total glutathione pool as measured by HPLC increased up to threefold. Therefore we suppose cadmium is detoxified by formation of Cd-$[GS]_2$ complexes in *P. patens*. This hypothesis was supported by *in vivo* labelling of GSH with monochlorobimane (MCB). MCB is a non-fluorescent, membrane-permeable dye which is conjugated by glutathione-S-transferases in a phase II reaction to form fluorescent, membrane-impermeable glutathione-S-bimane (GSB). These bimane conjugates can be quantified after separation by RP-HPLC. In contrast to the threefold increase in total glutathione the amount of GSH accessible to in vivo labelling with MCB was significantly reduced compared to non-stressed cells. The diminished GSH content accessible to MCB is consistent with the formation of Cd-$[GS]_2$ complexes in Cd^{2+} treated cells.

PO 4.13

Speciation of five arsenic metabolites in urine using high performance liquid chromatography and post column UV digestion coupled with hydride generation atomic fluorescence spectrometry (HPLC-UV-HGAFS)

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Arsenic and its compounds are known to cause several adverse health effects. Urinary As metabolites were used recently in epidemiological and environmental health studies as a means of assessing exposure to arsenic from drinking water. The concentrations of urinary As methylation are often found low enough (~ ng/ml) which require sensitive analytical methods involving speciation, identification and quantification of each individual As species. Arsenobetaine (AsB), arsenite (As^{III}), dimethylarsenic acid (DMA^{V}), monomethylarsonic acid (MMA^{V}) and arsenate (As^{V}) have been separated in one single chromatogram run using ion pairing reverse phase HPLC within 10 minutes. A mobile phase of pH 9 containing 20mM ammonium phosphate, 5mM TBAH and 4% ethanol was used. The eluent was further digested using an online post column UV digestion device at 200°C, followed by the sensitive detection of HGAFS which was able to provide detection limits of 0.4-0.8 ng/ml for all the arsenic species. The developed method provided a sensitive, robust approach for the monitoring of arsenic methylation in human urine samples.

Study of the protein-bound fraction of iron and platinum in biological samples by single-dimensional electrophoresis and laser ablation inductively coupled plasma mass spectrometry (LA ICP MS)

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In this work the analytical futures of single-dimension electrophoresis followed by LA ICP MS determination will be presented. Platinum and iron speciation was investigated for this purpose. Platinum complexes are now a well-established class of antitumor agents and play an important role in cancer chemotherapy. Since cancer chemotherapy is often associated with high toxic risks, and therapeutic drug monitoring based on and pharmacokinetic parameters may reduce toxicity and even enhance efficacy. Iron is recognized as an essential element in the living organisms. About one third of the total body iron is bound to storage proteins, primarily ferritin or hemosiderin in the liver, spleen, and bone marrow. About two third of the total body iron impact metabolic and enzymatic processes in living organisms. Iron plays an important and essential role as cofactors of proteins in biological systems. The absence or a deficit of iron in proteins results in deficiency diseases, but this metal can also catalyze cytotoxic reactions.

The investigation of metal-containing proteins is a new and challenging task in the proteomics field including the protein identification and the determination of the metal concentration, which requires sensitive analytical techniques.

Laser ablation inductively coupled plasma mass spectrometry (LA ICP MS) has gained increased popularity due to its ability to perform direct and almost non destructive analysis of solids. The direct multi-element determination of major, minor and trace elements in a variety of solids samples is possible. Moreover, with LA ICP MS the information on lateral distribution of selected elements and their isotopes on the surface and within a depth profiles in sub-surface domains could be collected.

LA ICP MS technique has been developed for the direct determination of iron and platinum speciation in human protein separated by single-dimensional gel electrophoresis.

Total concentration of both elements in biological samples (urine, serum, whole blood) was determined after sample digestion by an inductively coupled plasma mass spectrometry (ICP MS).

Quantitative determination of Cd-metallothioneins in prawn samples by HPLC-ICPMS

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Metallothioneins (MT) are ubiquitous, cysteine-rich proteins that have been ascribed various biological roles including involvement in metal detoxification processes. Earlier aquarium experiments with crabs exposed to cadmium showed that they produce a particular and unusual isoform at high exposure, and in a follow-up field study, this same MT isoform was identified as one of at least five MTs in the muscle of female coral prawns collected from a site naturally high in cadmium. These data suggested that the MT sub-isoforms in coral prawn may be formed in response to high Cd exposure, and hence it could be a selective biomarker of excessive, and toxic, Cd levels. The data on the prawn abdominal muscle so far show that several Cd-MT isoforms are present, and that the pattern of these MTs is related to the total concentration of cadmium.

We report studies on the development of an HPLC-ICPMS method for the quantitative determination of the various Cd-MTs in coral prawn. Effort has been directed to developing a simple extraction procedure to give intact Cd-MTs in an extract which is compatible with direct injection onto a reversed-phase HPLC column followed by selective detection of Cd and other metals with ICPMS. Factors investigated include extraction efficiency under various conditions, the stability of the MTs at each stage of sample preparation, and HPLC column recoveries of Cd. Besides parameters influencing the chromatographic separation, special attention has been paid to ICPMS instrumental factors influencing quantitative results.

We established a simple method for the quantitative determination of cadmium in sub-isoforms of metallothioneins separated with reversed phase chromatography using a methanol gradient. A novel approach to level the dependence of the response in ICPMS on the carbon content in the plasma was studied.

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