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Atomic Spectrometry Update: review of advances in elemental speciation

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Abstract

This is the eighth Atomic Spectrometry Update (ASU) to focus on advances in elemental speciation and covers a period of approximately 12 months from December 2014. This ASU review deals with all aspects of the analytical atomic spectrometry speciation methods developed for: the determination of oxidation states; organometallic compounds; coordination compounds; metal and heteroatom-containing biomolecules, including metalloproteins, proteins, peptides and amino acids; and the use of metal-tagging to facilitate detection via atomic spectrometry. The review does not cover fractionation, which is sometimes termed operationally defined speciation. As with all ASU reviews the focus of the research reviewed includes those methods that incorporate atomic spectrometry as the measurement technique. However, because speciation analysis is inherently focused on the relationship between the metal(loid) atom and the organic moiety it is bound to, or incorporated within, atomic spectrometry alone cannot be the sole analytical approach of interest. For this reason molecular detection techniques are also included where they have provided a complementary approach to speciation analysis. As in previous years, As and Se speciation continues to dominate the current literature and there has also been an increase in the number of publications concerning solid state speciation. This is presumably due to the increase in the number of synchrotron facilities available and a greater awareness of their potential for speciation studies.

Contents

1 Topical Reviews

This latest Update adds to that from last year[36/08284] and complements other reviews[36/01313], [36/05665], [36/06212], [36/10327],[ASU6] of analytical techniques in the series of Atomic Spectrometry Updates. Two *text books* have been published during the review period. Elemental speciation topics are featured throughout the Handbook of Mineral Elements in Food, whose emphasis is on both chemical analysis (of a very wide range of foodstuffs) and the regulatory and legislative framework within which the relevant scientists are operating. (*Handbook of Mineral Elements in Food, eds.* M. de la Guardia, M. Guàrdia, and S. Garrigues, John Wiley & Sons, New York, 2015)[36/20000]. Several of the chapters in a recent volume in Wilson and Wilson's Comprehensive Analytical Chemistry series entitled Chemical Imaging Analysis are concerned with the provision of elemental speciation information (F. Adams and C. Barbante, *Chemical Imaging Analysis*, Comprehensive Analytical Chemistry Vol. 69, ed. D. Barcelo, Elsevier, Amsterdam, 2015.)[36/20001].

Several reviews have also focused on aspects of *elemental imaging*. In two that are concerned with metals in biological materials, the reviewers point out that the determination of metal distribution,

concentration and flux defines our understanding of the fundamental cellular processes of development, health and disease. And that distribution of metals in biological samples is currently one of the most important issues in physiology, toxicology, pharmacology, and other disciplines where functional information about the distribution of metals is essential. The first review[36/09633] (51 references) adopts a tutorial approach with selected examples of the techniques, including MALDI, LA-ICP-MS, SIMS, targeted MS (coupled with the use of isotopically enriched materials), XRF, and micro particle induced X-ray emission. The review contains a substantial section on metal-specific fluorescent probes, and a discussion of the approaches to studies of metal-protein association in which REE nanoparticles are used as the tags, that includes the cyTOF MS system, a commercial apparatus that is capable of simultaneously resolving up to 100 individual isotopes. The reviewers also discussed the challenges of 3-D imaging and provided examples of *in vivo* measurement while maintaining the native biochemical environment. The review includes a discussion of the problems of changes induced by both sample preparation and by the measurement processes. The reviewers concluded (a) that biologists now have a comprehensive arsenal of analytical techniques at their disposal to address specific questions about metal biology, beyond simply determining where metals are located and at what concentrations, and (b) that establishing feasibility, practicality and sufficient access at the planning stage of an experiment is essential.

The second review[36/07484] (122 references with titles) provides a more comprehensive coverage that includes studies of the distribution of trace metals in tissues after a drug dose or ingestion of poison-containing metals. Speciation topics are distributed throughout the review, which includes substantial sections on LA-ICP-MS, SIMS and X-ray methods. The authors offered some suggestions for clarification of terminology as follows: imaging (of elements) is a test result showing distribution of the analyte on the all-over surface of the sample using an analytical technique that allows measurement in its picture mode; hence (bio)imaging is the imaging distribution of biochemical analytes (e.g. elements playing a biological role). On the other hand, mapping (of elements) would be a test result showing distribution of the analyte on the surface of the sample using an analytical technique that allows for distribution, point by point (mapping/raster mode). Researchers are also urged to include information about spatial resolution and analytical depth. It is concluded that although MS-based techniques are destructive, they have greater specificity and better detection capabilities than X-ray methods, which have the advantage of being non-destructive.

On the other hand, Wirtz et al.[36/12074] have reviewed *elemental imaging* by SIMS (152 references), covering a range of techniques from traditional 2D and 3D imaging to correlative microscopy. A tutorial approach is adopted to the explanation of the basic principles, with illustrative examples, and the review concludes with a discussion of approaches in which SIMS is combined with various high-resolution microscopy techniques, to provide higher sensitivity while achieving excellent spatial resolution by overlaying SIMS images with high-resolution images obtained from transmission electron microscopy, helium ion microscopy, and scanning probe microscopy. Recent advances in the application of LA-ICP-MS in the life sciences and environmental chemistry have been reviewed(175 references)[36/09852]. In addition to the imaging of various materials, the reviewers also deal with approaches to quantification and to the analysis of liquids

via the dried drop approach. It is clear that this latter method suffers from problems of repeatability with RSDs of up to 23% being reported.

While there appears to be a growing interest in the *application of nanoparticulate materials* in analytical chemistry, as indicated by several recent reviews of automated flow assays[36/10962], enhanced liquid-phase microextraction[36/08040] and elemental speciation[36/02737], there is still concern over the difficulty of making reliable measurements of the dissolution of metal-based nanomaterials in biological tissues. In a review (171 references, no titles or links) of this topic, Su and Sun[36/10202] examine procedures based on ICP-MS with particular reference to the limitations of currently available sample preparation procedures, the various differentiation schemes. They concluded that many existing sample preparation and separation techniques need improving in terms of size calibration and discrimination of dissolved ionic species by single-particle ICP-MS. In addition, they identified the need for new analytical methods for liberating residual nanomaterials and their released metal ion species without changing their original status. They also pointed to the role for complementary imaging techniques, such as transmission electron microscopy and X-ray spectroscopies, than can be applied with minimal sample destruction.

The role of elemental speciation analysis in characterizing *the interaction of metals and metalloids (and compounds containing such elements) with biological systems* has been the subject of several reviews. Harrington and Taylor[36/04912] discussed analytical approaches to investigating two "metal"-containing drugs, having first surveyed the more general need for the measurement of potentially harmful elements in biological fluids or tissues arising from the use of dietary supplements and toiletries as well as the consumption of active pharmaceutical ingredients (APIs). The two APIs highlighted are 4-(N-(S-glutathionylacetyl)amino) phenylarsenoxide (GSAO), which is under investigation for the treatment of solid tumours, and cis-diamminedichloroplatinum (II) (cisplatin), which is widely used for treatment of testicular, ovarian, bladder, head and neck cancers. For the study of this latter drug, the role of HPLC-ICP-MS was discussed, as well as the use of organic MS to characterize the API adducts with DNA. The central role of ICP-MS in providing metallic profile information in clinical and forensic toxicology has been reviewed[36/02477]. The 96 articles cited (with titles) cover applications to the analysis of whole blood, plasma, urine, hair, nail, biopsy and other tissue samples. There is relatively little in terms of elemental speciation in the review, other than a discussion of the role of isotope analysis, and a mention of the potential of the CZE-ICP-MS coupling for the determination of metallothioneins. However, three ASUs are highlighted as being of special note and considerable interest. In a review of elemental analysis in biotechnology, Han et al. illustrate a number of important applications by careful selection of relevant examples from the recent literature[36/02508]. The topics covered include the possibility of accurate absolute quantification even in complex matrices, elemental detection for chromatographic separation, the complementarity of inorganic and organic mass spectrometry, the multi-element capability and the capability of isotopic analysis. Some of the 52 articles selected are labeled as being of "special interest" and a subset of these are identified as of "outstanding interest." Both of these types of article feature, in the references section, a one- or two-sentence commentary indicating what is special.

Although not concerned directly with elemental speciation analysis, a review of the *effects of the*

introduction of organic solvents[36/09812] into inductively coupled plasmas deals with a number of topics of relevance. This review (173 references, with titles), which is part one of a two-part series, is divided into four sections: (a) the impact of organic matrices from aerosol generation on atomization, excitation and ionization processes, (b) the production and spatial distribution of molecular carbon species, (c) modifications of fundamental plasma properties, and (d) the resulting interferences. There is a substantial table listing enhancement effects, that includes many examples for elements that are typically the subject of speciation by separation in solvents that contain organics, such as As and Se. Part two, which has now been published,[A. Leclercq, A. Nonell, J. L. T. Torro, C. Bresson, L. Vio, T. Vercouter and F. Chartier, *Anal. Chim. Acta,* **885**, 57-91, 2015] deals with practical aspects, such as modified introduction devices, re-optimization of operating parameters, and quantification. The reviewers invent, but do not define, the term "hydro-organic" for a class of solvents. It probably means a solvent that consists of a mixture of organic solvents with water.

Elemental speciation involving *preconcentration and separation with quantification by AFS* has been reviewed[36/09493]. The 135 references (with titles) cited cover LLE, SPE, SPME, MAE, UAE and chemical vapor generation. Speciation applications are embedded throughout the review, which is devoted mainly to the determination of As, Hg, and Se compounds. There is a lengthy section devoted to extraction of species from solid samples, and the reviewers conclude by pointing to the potential for a further development in SPE procedures via advances in nanomaterials technology. Liu and Tang[36/02737] have reviewed the applications of nanoparticles in elemental speciation analyses, all of which are based on selective extraction and separation. The 62 articles (with titles) cited cover magnetic nanoparticles, carbon nanotubes, metallic oxide nanoparticles, nanostructured mixed oxides, and ion-imprinted polymer nanoparticles. The reviewers point to possible future applications of nanoparticles as chromatographic stationary phases and as pseudostationay phases in CZE.

2. Solid State Speciation

Almost all of the reports of elemental speciation in solid samples published in the current review period are of studies in which some, or all, of the analytical information has come from experiments performed with synchrotron radiation. The researchers have, therefore, booked time on an appropriate beamline and then taken the samples to an appropriate facility. Such work is rather difficult to evaluate in terms of the novelty of the analytical spectrochemistry, as developments in, for example detector capabilities, synchrotron beam brightness, or spectral data handling, are outside of the areas of expertise of the typical analytical atomic spectroscopist who is approaching elemental speciation analysis as a combination of extraction, separation, and quantification by atomic absorption, emission, fluorescence, or mass spectrometries. However, there seems to be little doubt that the range of spectrometric and imaging techniques available to a wide range of scientific disciplines is increasing and so researchers need to be aware of the information that is available from these kinds of facilities. For a comprehensive critical review of developments in synchrotron radiation XRF (SRXRF), readers are referred to the fifth Atomic Spectrometry Update in the annual cycle of ASU reviews (Review of advances in and applications of X-ray fluorescence spectrometry)[ASU#5]. Although notionally restricted to XRF techniques, the review does also

include a variety of other techniques, such as EXAFS and XANES, as researchers often conduct multiple experiments at the same facility to maximize the amount of information that is collected in the time allocated.

Many of the papers appearing in the current review period are concerned with obtaining *elemental speciation information for biological systems*. Two reviews of a range of bioimaging and mapping techniques,[36/07484][36/09633] already discussed, are obviously concerned with solid samples, and feature techniques other than synchrotron based X-ray methods, such as LA-ICP-MS. Although the emphasis is on obtaining spatially resolved information, speciation topics are featured throughout the reviews. Sugiyama et al.[36/03137] examined trace metal elements in oral mucosa specimens by using SRXRF, PIXE, and XAFS, pointing out that the analyses could be carried out without damage on conventional histopathological specimens and therefore, these techniques could be applied to the determination of accumulated trace metal elements in biopsy specimens from the oral mucosa. They were able to identify metal contamination from chips from the stainless steel microtome blade used to prepare samples, and from nickel silver formerly used in both dental restorations and oral surgical instruments. In an examination of the distribution and speciation of Br in mammalian tissues and fluids, to provide further insights into the role and function of this element in biological systems,[36/07264] XRF imaging and XAS were applied together with ICP-MS. Blood (whole and serum) from several animal models was examined, as were tissues from a number of marine organisms. A library of 9 Br-containing compounds was obtained, and the researchers concluded that that the major form of Br in all samples analysed was bromide. Other researchers have examined the speciation of V derived from four antidiabetic drug candidates in the blood of rats and in bovine serum[36/09829]. Speciation information was obtained from XANES, on freeze dried samples, whereas the distribution of V between red blood cells and plasma was determined by ETAAS. The researchers found that the spectra of V compounds in red blood cells were independent of the nature of the initial V complex, and were best fitted by a combination of V^{IV} carbohydrate (2-hydroxyacid moieties) and/or citrate (65-85%) and V^{V} protein (35%), and they concluded that red blood cells act as V carriers to peripheral organs. Moving to the plant kingdom,[36/00600] the speciation and localization of Zn in the hyperaccumulator *Sedum alfredii* (a variety of stonecrop) by EXAFS and micro-XRF has been studied. Fresh plant tissues were ground under liquid nitrogen and pressed into 2-mm path length Lucite [poly (methyl methacrylate)] sample holders with Kapton [poly (4,4'-oxydiphenylene-pyromellitimide)] tape windows cooled in liquid nitrogen. It was found that the plant complexes Zn with oxygen donor ligands for storage in leaves and stems, and that the dominant chemical form of Zn in leaves was a complex with malate, the most prevalent organic acid in the leaves. In the stems, Zn was mainly associated with malate and cell walls, whereas Zn-citrate and Zn-cell wall complexes dominated in the roots. Finally in a study of the distribution and associations of organobromine compounds in soils,[36/00606] a variety of synchrotron radiation based spectromicroscopies were used, including FT-IR absorption, micro XRF and XANES. Decabromodiphenyl ether (BDE-209) and tetrabromobisphenol A (TBBPA), two typical brominated organic contaminants, were selected as the organobromine compound probe molecules and a brown soil (classified as an Alfisols is the USDA taxonomy, with an organic carbon content of 1.98%), which was spiked with the compounds at a concentration of 50 mg kg^{-1} (as Br). The sample preparation consisted of picking intact particles (100 − 500 μm) from the water-

saturated spiked soil samples with superfine tweezers under a light microscope. The selected particles were frozen at − 40 ° C and directly sectioned without embedding. Thin sections (4− 8 µm in thickness) were cut at − 20 ° C using a cryomicrotome and transferred to infrared-reflecting microscope slides. To ascertain the speciation of Br and the nature of organic compound forms and the clay minerals in the soil, Br K-edge XANES and FT-IR spectra from the hotspots of the examined regions were collected. The Br K–edge XANES spectra provided evidence for significant accumulation of BDE-209 in the hotspots in the soil, as there was no detectable Br (inorganic or organic) signal in the unspiked soil. The O− H stretching vibration at 3621 cm^{-1} was assigned to Illite, whose presence as the main clay mineral was confirmed by X-ray diffraction analysis.

Three applications to the *elemental speciation as a function of time* have been described. In the first, discussed in more detail in section ?.4, the darkening of chrome yellow pigments in paintings by Vincent van Gogh was studied by full spectral XANES imaging[36/05290]. In the second, a method for quantitative mapping of the Mn speciation inside partially corroded historical windowpanes based on SR-XANES was discussed[36/05351]. Glasses were also examined by XRF microscopy, and the calibration of such Mn oxidation state maps based on the combo method, described by the researchers as “a fairly reliable way to determine the oxidation state”, was described in detail. The researchers also discuss the problems of photoreduction during the measurement process. As the darkening is usually the result of the oxidation of Mn^{II} to Mn^{III} and Mn^{IV}, the effectiveness of reducing treatments on 14th century glass, originating from Sidney Sussex College (Cambridge, UK), suffering from “Mn browning” was evaluated. The most effective of the treatments consisted of placing cotton soaked in 5% w/w hydroxylamine hydrochloride in contact with the glass for 24 h. In the third study, the Cr speciation in solid waste material and eluates was determined by SR-XAS[36/08153], which allowed the determination of mobilizable Cr^{VI} directly in the solid materials and in the alkaline eluates, at concentrations below 500 µg L^{-1}. This work is also discussed in more detail in section ?.5

3. CRMs and Metrology

An *SI traceable method for the absolute quantification of S containing proteins*, using an exact matching double isotope dilution ICP-MS method, has been reported this year[36/11634]. The method was applied to the analysis of a candidate human growth hormone (hGH) reference material, CRM 111-31-01A, produced by the Korea Research Institute of Standards and Science (KRISS). Initially, a ^{34}S enriched sulfate spike was added to the hGH and an HNO_3/H_2O_2 MAE procedure used to solubilise the sample. A separate blend of the ^{34}S enriched spike material and NIST SRM 3154 sulfur CRM was also prepared to act as the calibration standard during the ID-ICP-MS measurements. Subsequently, the ^{32}S:^{34}S isotope amount ratio in the samples and calibration blends was measured using a triple quadrupole ICP-MS instrument operated in mass shift mode. In this mode oxygen was utilised as the reaction gas, producing $^{32}S^{16}O^+$ and $^{34}S^{16}O^+$ ions from the measurement of which the ^{32}S:^{34}S isotope amount ratio in the hGH and calibration blends was calculated. This part of the measurement procedure was validated by the use of NIST CRM 2389a, which is certified for methionine and cysteine content, with the found value for total S being in good agreement with the certified values converted to that for the total S content. The purity of the hGH

sample, in terms of the presence of other S containing species, was assessed using a standard addition approach and SEC-ICP-MS. Finally, the impurity content (0.022 ± 0.021 mg/kg as S) was subtracted from the total S content (18.88 ± 0.77 mg/kg as S) to give the mass fraction of the hGH in the original sample (18.86 ± 0.73 mg/kg as S, 1.859 ± 0.076 g/kg as hGH). This value compared favourably, with a lower relative expanded uncertainty, with the hGH mass fraction of 1.89 ± 0.19 g/kg which was determined by a previously reported ID-LC-MS/MS method using four isotopically labelled amino acids and acidic hydrolysis. The authors conclude that the approach used has the potential to be considered as a primary method for the characterisation of pure, S containing, biological and other organic standards.

There have been no other reports of new CRMs certified for individual chemical species published during the period covered by this review.

4 Elemental Speciation Analysis

4.1 Antimony

There have been few advances in Sb speciation studies this year. The speciation of Sb in soils and sediments has been reported by a number of groups, although studies are often site specific and use established methodologies with little novel analytical development. Some however are of interest, if only for the scale of the contamination. In one such study[36/01080], indigenous plants and associated soils were sampled at the Xikuangshan Sb mine in China revealing levels in the range 441-1472 mg kg^{-1}. The presence of four species of Sb in the soils and plants, Sb^{III}, Sb^{V}, TMSb and an unknown species reported to contain Sb^{V} were detected using HPLC-ICP-MS.

The determination of *Sb species in natural waters* continues to attract attention. A study by Gao *et al*.[36/09573] has reported a novel approach using photochemical vapour generation (PVG) coupled with ICP-MS for detection. A flow-through photochemical reactor capable of subjecting the samples to deep-UV (185 nm) radiation was used, and the generation efficiency found to be independent of whether Sb^{III}, Sb^{V}, or organometallic species ($TMSb^{V}$ dibromine, $TMSb^{V}$) were present, eliminating the shortcoming of Sb species dependent sensitivity encountered during direct solution nebulization by ICP-MS. Potentially severe matrix effects from seawater was efficiently eliminated by using a mixture of 5% (v/v) formic and 15% acetic acids (v/v) as the photochemical reductant. A LOD of 0.0006 ng g^{-1} based on external calibration was obtained (0.0002 ng g^{-1} for IDA), yielding a 15-fold improvement over that for direct solution nebulization. Accuracy was demonstrated by analysis of two water CRMs (SLRS-6 and NIST 1640a) with good results obtained. In addition, spike recoveries of 100.6 +/- 5.5% and 100.8 +/- 3.8% (n = 3) were obtained for NASS-6 and CASS-5 seawater CRMs, respectively, since no certified values for Sb has been established for these materials.

A non-chromatographic automated system for the speciation and determination of inorganic and TMSb exploiting multisyringe FIA with HG and AFS has been described[36/06765]. A cationic minicolumn was used to retain the methylated forms of Sb. Optimization was achieved using a multivariate Box-Behnken design and a multiple response strategy to facilitate the quantification of Sb using calibration with aqueous standards. The method was applied to the determination of Sb^{III},

Sb^{V} and TMSb in drinking, surface and ground water samples collected in the Balearic Islands, Spain, with LOD values of 0.03 µg L^{-1} for Sb^{III} and Sb^{V} and 0.13 µg L^{-1} for TMSb.

Table 1 shows selected examples of other application of Sb speciation presented in the literature during the time period covered by this ASU.

Table 1 Applications of Speciation Analysis: Sb

Analyte species	Technique	Matrix	Sample treatment	Separation	LOD	Validation	Reference
Sb^{III}, Sb^{V}	FAAS	Beverages, serum, plasma	Acid digestion (nitric, sulfuric, perchloric)	CPE, Sb^{V} with Victoria Pure Blue + Triton X-114, not clear how selectivity over Sb^{III} was achieved	0.2 µg L^{-1}	TM 25.3 (a low level fortified standard for trace elements), NIST 1643e (trace elements in water), Serornorm (trace elements whole blood), spike recovery	36/02146
Sb^{III}, Sb^{V}	HG-AAS (continuum source)	Sediment	USE (20 min 70 °C) 200 mg + 5 mL 7.2 M HCl + 1 mL of 5% sodium citrate	HG, Sb^{III} in presence of citrate, total Sb after reduction with KI and ascorbic acid	0.7 µg kg^{-1}	MESS-2 (marine sediment), PACS-2 (marine sediment)	36/07787
Sb^{III}, Sb^{V}	ET-AAS	plastic	10 g sample extracted with 400 mL leaching solution (hardness	DLLME, Sb^{III} with 1,2,6-hexanetriol trithioglycolate in CCl_4, Sb^{V} reduced with 0.3% L-cysteine +	0.03 µg L^{-1}	GSB 07-1376-2001(environmental water), spike recovery	36/07352

			100 mg L^{-1} pH 8 + 2 mg L^{-1} active chlorine 24 h.	0.002 M tartaric acid (70 °C, 2h)			
Sb^{III}, Sb^{V}	ICP-OES	Bottled water	None	HG, Sb^{III} in presence of citrate, total Sb after reduction with KI	0.05 – 0.1 µg L^{-1}	Spike recovery	36/10637
Sb^{III}, Sb^{V}, TMSb	AFS, post column HG	Soil and sediment	200 mg extracted with ammonium tartrate (60 °C in dark), filtered (0.22 µm)	HPLC, AE 0.3 M ammonium tartrate at pH 3.5 + 5% methanol	0.1 – 0.4 µg L^{-1}	GBW07407 (soil) and GBW07301a (sediment), spike recovery	36/07909
Sb^{III}, Sb^{V}, TMSb	AFS, post column HG	blood	3 mL cytoplasm or plasma + 900 µL 0.1 M EDTA + 3 mL saturated $(NH_4)_2SO_4$ centrifuged (5000 rpm 45 min. SPE on C18, elute 3 mL EDTA filtered (0.2 µm	HPLC AE Hamilton PRP X-100 (100 mm x 4.1 mm) 0.020 M EDTA	Not given	None used	36/02761

Sb^{III}, Sb^{V}	ICP-MS m/z 121	Water	Filtered (0.45 µm)	Magnetic SPE. Sb^{III} extracted at pH 5 as APDC complex on octyl-immobilized silica coated magnetic Fe_3O_4. Sb^{III} and Sb^{V} extracted at pH 2	0.001 – 0.004 µg L^{-1}	GSB 07-1376-2001 (environmental water), spike recovery	36/03865

4.2 Arsenic

Two *reviews on arsenic speciation* have recently been published. The measurement of As species in environmental, biological fluids and food samples by HPLC-ICP-MS and HPLC-HG-AFS has been covered by Maher *et al.*[36/10876]. The review focuses on the integrity of As species during collection, storage, sample preparation as well as measurement, and the paper includes a lot of useful information for those interested in As speciation. For example, most samples can be stored frozen (-20^{o}C), but the stability of water and sediment samples is matrix dependent and depends on preservation technique applied. Arsenic cannot be extracted from samples using a single set of conditions, but must be optimised for each sample type. Methanol-water mixtures with microwave heating are commonly used to extract polar As species from tissues while As-lipids required a non-polar solvent. Dilute acid can be used to increase the efficiencies of extraction of hard to extract tissue As species. Freeze drying is suitable for the drying of biotic material while sediments should not be dried before analysis. Extraction efficiencies are critically dependent on particle size. Polar As species have a wide variety of ionic characteristics thus complimentary chromatographic approaches utilising ion-exchange or RP columns with modifiers are needed to separate all the As species. Arsenic-lipids require the use of a RP column and gradient elution with high concentrations of organic solvents and require compensation for carbon enhancement effects in the ICP-MS. Finally, to ensure accurate results, mass balances and extraction and column recoveries need to be determined at all steps, and methods need to be evaluated using As spikes and CRMs to provide a means of assessing the quality of results. A more focused review of the use of coupled techniques for As speciation in food and drinking water has also been published[36/01889]. The speciation steps (sample preparation, species speciation and detection) most commonly used for the determination of As in food were described, together with the coupling of GC and HPLC to AAS, AFS, ICP-AES and ICP-MS. Some of the less utilised techniques for As speciation were also included in the review.

The use of HPLC-ICP-MS has become routine for speciation studies and the technique of choice for many applications. However, this year has seen the first report of *triple quadrupole ICP-MS (ICP-QQQ) for As speciation*[36/07461]. The approach effectively modified exising methods using a short, small particle size column (Hamilton PRP-X100 5 µm, 4.1 x 50 mm) with injection volumes up to 20µl. The column was directly interfaced with the ICP-QQQ-MS. Oxygen was used as the reaction gas with detection of the resultant AsO at m/z 91. Using H_2O_2 in the extractant to oxidize As^{III} to As^{V} it was possible to separate AB from DMA, MMA and As^{V} in 1.5 minutes. Separation of 6 As species in the CRM NIST 2669 (standard urine) was achieved in under 3.5 minutes using a gradient elution. The LODs for a 20ml injection volume based on 3σ baseline noise were ca.10 ng/L for AsC, AsB, DMA, MMA and 15 ng/L for As^{III} and As^{V}.

The *ultra-trace determination of As species in environmental samples* such as waters, food and biological samples continues to be of interest. A method using a modified aluminium oxide nanoparticle sorbent and ETAAS detection after optimization using fractional factorial design method has been reported[36/09642]. Adsorbed As was eluted from the sorbent with 1 M hydrochloric acid. Pre-concentration factors up to 750 were achieved depending on the sample volume, and interference studies showed the method to be highly selective. The calibration plots were linear in the 5.0 to 280 ng L^{-1} and 8.0 to 260 ng L^{-1} concentration ranges for As^{III} and total As respectively. The LOD values (calculated for S/N ratios of 3) were 1.81 and 1.97 ng L^{-1} for As^{III} and total As respectively. Further reports on the use of multiwalled carbon nanotubes for preconcentration of As^{V} species have been reported. As^{V} was selectively sorbed onto a nanotube column at pH 9.5 in the presence of 2-(5-bromo-2-pyridylazo)-5-diethyl amino phenol (5-Br-PADAP)[36/00009]. The adsorbed species were then desorbed with 1mL of 2.0M HNO_3 prior to detection by ETAAS. Experimental parameters including pH, sample volume and flow rate, type, volume and concentration of eluent that influence the recovery of the As^{V} species were optimised. Under the optimised conditions, the calibration curve was linear in the range of 0.2-10.0 µg L^{-1} with an LOD of 0.016 µgL^{-1}. The RSD for seven replicate determinations at 1.0 µg L^{-1} level of As was 6.69%. The proposed method was successfully applied to the determination of As in water samples and the CRM NIST RSM 1643e. The use of *ionic liquid-assisted multiwalled carbon nanotube-dispersive µSPE* for the determination of iAs species in garlic samples by ETAAS has been reported by Grijalba *et al.*[36/09609]. Trihexyl(tetradecil)phosphonium chloride was used to form an ion pair with the arsenomolybdate complex obtained by reaction of As^{V} with molybdate ion prior to 1mg of multi-walled carbon nanotubes being dispersed for As^{V} extraction. The supernatant was separated by centrifugation. The multi-walled carbon nanotubes were then re-dispersed with tetradecyltrimethylammonium bromide surfactant and ultrasound before direct injection into the ETAAS for As determination. An extraction efficiency of 100% and a pre-concentration factor of 70 was obtained with 5 mL of garlic extract. The LOD was 7.1 ng L^{-1} and the RSDs for six replicate measurements at 5 µg L^{-1} of As were 5.4% and 4.8% for As^{III} and As^{V}, respectively. The preconcentration and *separation of trace As^{III} and Sb^{III} by carbon nanofibers* loaded with APDC prior to ICP-MS determination has also been reported[36/09440]. The effects of pH, sample flow rate and volume, elution solution and interfering ions on the preconcentration and separation of the analytes were examined. The adsorption capacity of carbon nanofibers-APDC for As^{III} and Sb^{III} was 3.6 mg g^{-1} and 2.8 mg g^{-1}, respectively. Under the optimum conditions, the LOD (3σ) of the method was 0.065

ng mL-1 of As^{III} and 0.012 ng mL^{-1} of Sb^{III}, and the RSD was 3.1% and 5.4%, respectively (n=9, 1.0 ng mL^{-1}). The method was applied to the determination of trace As^{III} and Sb^{III} in natural water samples with recoveries of 95.3-104%. In order to validate the method, a CRM of human hair (GBW 07601) was analysed, and the determined values were in good agreement with the certified values. Speciation of iAs in drinking water after in situ pre-concentration with miniature SPE disks has been reported[36/03706]. The method involved the simultaneous collection of As^{III} and As^{V} using 13 mm SPE disks. The removal of Pb^{II} from the sample water was first conducted to avoid the overlapping PbLα and AsKα spectra on the XRF spectrum. To this end, a 50 mL aqueous sample (pH 5-9) was passed through an iminodiacetate chelating disk. The filtrate was adjusted to pH 2-3 with HCl, and then APDC solution was added. The solution was passed through a hydrophilic PTFE filter placed on a Zr and Ca loaded cation-exchange disk at a flow rate of 12.5 mL min^{-1} to separate As^{III}-PDC complex and As^{V}. Each SPE disk was affixed to an acrylic plate using adhesive tape, and then examined by WDXRF. The LOD values of As^{III} and As^{V} were 0.8 and 0.6 µg L^{-1}, respectively. The proposed method was successfully applied to screening for As in spring water and well water.

The determination of As in specific foods has once again predominated in the literature. The use of *organoarsenicals in the poultry industry*, the analytical methods available for quantifying organic As, the fate and transport of organoarsenicals in environmental systems, and toxicological concerns associated with these chemicals has been reviewed[36/02793]. The authors identify a need for more emphasis on the less researched compounds such as arsanilic acid, carbarsone, and nitarsone. However, the determination of As residues in chicken meat following the use of feed containing the most common organoarsenic compound Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) has continued to attract attention. The enzyme-enhanced extraction of As species followed by separation using AEC and simultaneous detection with both ICP-MS and ESI-MS has been reported by Liu et al.[36/10853]. Several proteolytic enzymes (bromelain, papain, pepsin, proteinase K, and trypsin) were compared with a water/methanol extract. When using papain, 10 As species were extracted and detected, as compared to 8 As species in the water/methanol extract. The overall extraction efficiency was also improved using a combination of ultra-sonication and papain digestion. The LODs were in the range of 1.0-1.8 mg arsenic per kg chicken breast meat (dry weight) for seven As species: AsB, As^{III}, DMA, MMA, As^{V}, Roxarsone, and N-acetyl-4-hydroxy-m-arsanilic acid (NAHAA). Analysis of breast meat samples from six chickens receiving feed containing Roxarsone showed the presence of AsB (107 +/- 4 mg kg^{-1}), As^{III} (113 +/- 7 mg kg^{-1}), As^{V} (7 +/- 2 mg kg^{-1}), MMA (51 +/- 5 mg kg^{-1}), DMA (64 +/- 6 mg kg^{-1}), Roxarsone (18 +/- 1 mg kg^{-1}), and four unidentified As species with concentrations in the range 1-10 mg kg^{-1}. Response surface methodology has been applied to optimize the parameters for microwave-assisted extraction of six As species (As^{III}, As^{V}, MMA, DMA, p-arsanilic acid, and Roxarsone) from chicken tissues using a HPLC-ICP-MS[36/11201]. Once optimised for the effects of extraction medium, solution pH, liquid-to-solid ratio, and the temperature and time of microwave-assisted extraction, the method had good extraction efficiency for total As in the spiked and nonspiked chicken tissues;104.0 +/- 13.8% and 91.6 +/- 7.8%, respectively (except for the ones with As contents close to the LOQ). The LOQ values (S/N = 10) for As^{III}, As^{V}, DMA, MMA, p-arsanilic acid, and Roxarsone in chicken tissues using this method were 0.012, 0.058, 0.039, 0.061, 0.102, and 0.240 mg kg^{-1} (dry weight), respectively. A two stage instrumental approach, HPLC-ICP-MS in conjunction with ESI-MS-MS, been used to has been used

for the identification and quantification of eight As species (AB, As^{III}, As^{V}, MMA, DMA, 3-amino-4-hydroxyphenylarsonic acid, N-acetyl-4-hydroxyphenylarsonic acid (N-AHPAA), and Roxarsone) in chicken livers[36/00762]. The simultaneous detection by both ICP-MS and ESI-MS from the same HPLC separation allowed for comparison of peaks in both ICP-MS and ESI-MS chromatograms. Thus, fluctuations in retention times were overcome which prevented misidentification of co-eluting compounds, as demonstrated in this study by the identification of two possible metabolites of Roxarsone, N-AHPAA and 4-amino-phenylarsonic acid (4-APAA). The identity of N-AHPAA was confirmed by HPLC separation with simultaneous As-specific detection by ICP-MS and multiple reaction monitoring by ESI-MS. Although an As-containing compound had identical retention time as 4-APAA in the HPLC-ICP-MS chromatogram, it was ruled out as 4-APAA from the simultaneous detection by ESI-MS. The biodegradation of Roxarsone by bacteria in underground water following excretion by chickens, and the toxicity of the resulting compounds has been studied[36/08465]. The bacterial communities of microcosms were characterized by PCR- denaturing gradient gel electrophoresis (DGGE). Roxarsone degradation was measured by HPLC-HG-AAS. The results showed that microcosms of underground water with nutrients degraded 90 and 83.3 % of the Roxarsone under anaerobic and aerobic conditions, respectively, and that microcosms without nutrients degraded 50 and 33.1 % under anaerobic and aerobic conditions, respectively. Microcosms including nutrients showed more Roxarsone conversion into toxic iAs species. The authors conclude that using underground water from areas where Roxarsone containing manure is used as fertilizer might be a health risk.

As in previous years, interest in the *determination of As speciation in rice* (Oryza sativa L.) has resulted in a large number of publications this year. A review of the strategies available for sample preparation prior to spectrometric measurements for the determination and speciation of As in rice has been published[36/04292]. The review considers 76 papers published over the last 15 years and covers both cooked and uncooked rice as well as quality assurance and quality issues. Arsenic species in rice and rice cereal have been determined using CE-ICP-MS[36/05451]. An enzyme (α-amylase) assisted water-phase microwave extraction procedure was used to extract DMA, MMA, As^{III}, and arsenate As^{V} from the rice matrices. The addition of the enzyme during the extraction process reduced the sample viscosity, which allowed an increased injection volume and an enhanced signal response. A pH of 11 and an applied voltage of 20kV was used to complete the electropherograms within 10 minutes. The LOD values were in the range 0.15-0.27 ng g^{-1}. Rice CRMs SRM 1568b and CRM 7503-a were used for validation. The role of rice nodes in As storage and distribution has been studied using SR-μXRF[36/09443]. The incident x-ray energy was set at 12.4keVand the XRF spectra collected using a Si drift detector. The results suggest that rice nodes serve as a filter restricting As^{III} distribution to the grain with As preferentially accumulating in the phloem within different types of vascular bundles. Furthermore, the silic acid/ As^{III} transporter Lsi2 plays a role in As^{III} distribution in rice nodes and phytochelatins are important compounds for As^{III} storage in the nodes.

The oxidation of As^{III} can be accelerated by bacterial catalysis, but the effects of the accelerated oxidation on arsenic toxicity and translocation in rice plants are poorly understood. Yang et al.[36/10376] have investigated how the As^{III}-oxidizing bacterium *Brevibacillus laterosporus*,

influences As^{III} toxicity and translocation in rice plants. Rice seedlings of four cultivars, Guangyou Ming 118 (GM), Teyou Hang II (TH), Shanyou 63 (SY) and Minghui 63 (MH), were inoculated with or without the bacterium and grown hydroponically with As^{III} to investigate its effects on As toxicity and translocation in the plants. The percentage of As^{III} oxidation in the solutions with the bacterium (100%) were all significantly higher than those without (30-72%). The addition of the bacterium significantly decreased As^{III} concentrations in SY root, GM root and shoot, while increased the As^{III} concentrations in the shoot of SY, MH and TH and in the root of MH. Furthermore, the As^{III} concentrations in the root and shoot of SY were both the lowest among the treatments with the bacterium. The results indicate that As^{III} oxidation accelerated by *B. laterosporus* could be an effective method to alleviate As^{III} toxicity in rice seedlings. The interactive effects of Hg and As on their uptake, speciation and toxicity in rice seedling has been reported[36/01861]. A hydroponic experiment was conducted incorporating exposure to As^{III} (0.1, 0.5 or 2.5 mg L^{-1}) and Hg (0.05, 0.25 or 1.25 mg L^{-1}) over 14 days. Arsenic speciation was conducted by HPLC-ICP-MS. Rice was much more effective in taking up Hg than As and sequestered both in the roots. As and Hg reached 339 and 433 mg kg^{-1}respectively in the roots, and 48.5 and 16.1 mg kg^{-1} respectively in the shoots with the highest dosing. Though Hg inhibited As uptake and translocation, it enhanced As^{III} toxicity to the rice seedling. Arsenite (54-100%) and iHg (100%) were the predominant forms in the plant.

Several research groups have reported on *risk assessment and dietary exposure* calculations for As. In a European study[36/00102], drinking water, and dietary exposure to iAs via rice and seaweed have been investigated, particularly with respect to imported products. The bioavailability of iAs was measured in in vitro digestion experiments. The data indicate that the bioavailability of iAs is similar for rice and seaweed. The calculated dietary intake for specific EU populations varied between 0.44 and 4.51 µg kg^{-1} bw day^{-1}. Levels of As species in Australian and imported rice (n=36) have also been evaluated using HPLC-ICP-MS by Tinggi et al.[36/08745]. The study also assessed the daily intake of total As from diets of healthy children (n=15). A wide variation in the total As levels (range: <0.05-0.42 mg kg^{-1}) in Australian and imported rice was found. Australian rice levels were relatively high, with the mean level (0.25 +/- 0.08 mg kg^{-1}, n=7) of DMA being considerably higher than that of As^{III} (0.07 +/- 0.03 mg kg^{-1}, n=7). Children's daily intakes of total As varied widely, ranging from 1.7 to 31.2 (11.5 +/- 8.9 µg day^{-1}), which was comparable to other countries. Seaweed based dietary supplements have been further studied by Avula et al.[36/06014] to provided data for dietary exposure estimates. Solvent extraction with sonication and microwave extraction using various aqueous and aqueous/organic solvent mixtures were evaluated prior to using anion exchange HPLC-ICP-MS for the determination of species. The six As species (As^{III}, As^{V}, MMA, DMA, AB, and AC) were separated in approximately 8 min using gradient elution. The LOD values for all six compounds were in the range of 10-15 ng mL^{-1}. The most abundant As species found were As^{III} and As^{V}. The sum of iAs species present in the dietary supplements ranged from 1.2 to 31 µg day^{-1}. A recent study has also shown that Westernized diets lower As gastrointestinal bioaccessibility, but increase microbial As speciation changes in the colon[36/03412]. An in vitro gastrointestinal method (IVG) was used to evaluate how an Asian type diet (fiber rich) and a Western type diet (fat and protein rich), differ in their capability to release iAs^{V} and DMA^{V} from a rice matrix following gastrointestinal digestion. A dynamic gut simulator was also used to investigate whether diet background affects As metabolism by gut microbiota in a colon environment. Arsenic speciation was

determined by HPLC-ICP-MS. The Asian diet background resulted in a larger As bioaccessibility (81.2%) than a Western diet background (63.4%). However, incubation of As contaminated rice with human colon microbiota in the presence of a Western type diet resulted in more of the hazardous As species, monomethyl arsonite and monomethylmonothio arsonate, to be formed after 48 h. The authors conclude that dietary background is a crucial parameter to incorporate when predicting bioavailability with bioaccessibility measurements and when assessing health risks from As following oral exposure. The iAs in starchy roots, tubers, and plantain as part of an assessment of cancer risk of sub-Saharan African populations has been reported[36/04638]. The iAs in these crops was separated and preconcentrated by SPE prior to using HG-AFS. Overall, iAs in these crops ranged from 0.9 to 14.1 ng g^{-1} wet weight. The long-term cancer risk associated with iAs intake from these crops was assessed by Monte Carlo simulation based on iAs concentrations and historical consumption and population data. For 19 consuming sub-Saharan African countries, life-time cancer risk was low.

Ion-pair chromatography coupled ICP-MS has been evaluated and for the *determination of As species in fruit juice and fruit drink products*[36/02034]. A Prodigy^{TM} 3µ ODS-3, 2.0 mm x 150 mm column and a mobile phase consisting of 5 mmol l^{-1} malonic acid, 3 mmol l^{-1} of tetrabutylammonium hydroxide solution and 5 % methanol were used to achieve the As speciation. After demonstrating the method was sensitive, accurate and fit for purpose, and it was applied to analyse 96 fruit juice and fruit drink samples. Speciation analysis of the 96 fruit juice and fruit drink products revealed that iAs was the major species found in the majority of juice products tested in the study. The concentration of inorganic arsenic (sum of As^{III} and As^{V} ranged from < LOQ to 23.9 µg l^{-1}, with a mean value of 3.57 +/- 4.42 µg l^{-1}. DMA was found in 32 of the samples (mean 0.30 +/- 0.55 µg l^{-1}) and MMA was found in 26 of the samples (mean value is 0.30 +/- 0.71 µg l^{-1}).

Methods to extract As from plants containing varying levels of As for speciation studies have been reported[36/07155]. The plants used were an As hyperaccumulator, *Pteris vittata,* with As in the range 459-7714 mg kg^{-1}, rice seedling at 53.4-574 mg kg^{-1}, and tobacco leaf at 0.32-0.35 mg kg^{-1}. The four methods included heating with dilute HNO3, sonication with phosphate buffered solution, methanol/water, and ethanol/water, with the As species being determined using HPLC-ICP-MS. The ethanol/water method produced the most satisfactory extraction efficiency for *Pteris vittata* (80% for the roots and >85% for the fronds) without changing As species. A lower extraction efficiency from *Pteris vittata* roots was attributed to the dominance by As^{V} (82%) while As^{III} dominated in the fronds (89%). The ethanol/water method used a sample:solution ratio of 1:200 (0.05 g:10 mL) with 50% ethanol and 2 h sonication. Based on different extraction times (0.5-2 h), ethanol concentrations (25-100%) and sample:solution ratios (1:50-1:300), the optimized ethanol/water method used less ethanol (25%) and time (0.5 h for the fronds and 2 h for the roots). Extraction efficiencies for tobacco leaf (78-92%) and rice seedlings (70%) were obtained using the optimised method, which was better than the other three methods. Arsenic species in tobacco products have also been studied by Campbell *et al.*[36/01316]. Fourteen samples of tobacco were studied spanning a wide range of concentrations in samples from different geographical regions. The samples included CRM materials and cigarette products. The As species were extracted from powdered tobacco samples using a nitric acid microwave digestion procedure. The As species in the

extracts were determined using HPLC-ICP-MS. The concentrations of total iAs species ranged from 144 to 3914 µg kg^{-1}, whilst DMA ranged from 21 to 176 µg kg^{-1}, and MMA ranged from 30 to 116 µg kg^{-1}. The percentage of species eluted compared to the total As extracted ranged from 11.1 to 36.8% suggesting that much of the As (possibly macro-molecules, strongly complexed or in organic forms) do not elute from the column. However, the dominance of iAs species is a marked feature of the diverse range of tobaccos selected for study. Such consistency is important in the context of a WHO expert panel recommendation to regulate tobacco crops and products using total As concentrations. An investigation of the accumulation of As in and on roots of *Zea mays* (maize) and *Helianthus annuus* (sunflower) using SR-µXRF has been reported[36/08540]. Plant and soil samples were collected from two field sites in the Hetao Plain (Inner Mongolia, China) which have been regularly irrigated with As-rich groundwater. The results showed that average As concentrations in the roots (14.5-27.4 mg kg^{-1}) covered a similar range to the surrounding soil, but maximum root As concentrations reached up to 424 mg kg^{-1} in *H. annuus* and 1280 mg kg^{-1} in *Z. mays*. The results also revealed that the As had mainly accumulated at the outer rhizodermis along with Fe. The authors conclude that thin crusts of Fe-(hydr)oxides cover the roots and act as an effective barrier to As, similar to the formation of Fe plaque in rice roots. In contrast to permanently flooded rice paddy fields, regular flood irrigation results in variable redox conditions within the silty and loamy soils of the study site which fostered the formation of Fe-(hydr)oxide plaque on the root surfaces.

The number of studies looking at *As in edible fungi* have increased this year. The mushrooms of 46 different fungus species (73 samples) over a diverse range of phylogenetic groups were collected from Canadian grocery stores and background and arsenic-contaminated areas by Nearing et al.[36/01784]. Total arsenic was determined using ICP-MS, and As speciation was determined using HPLC-ICP-MS and XAS. The major As compounds in mushrooms were found to be similar among phylogenetic groups, and AB was found to be the major compound in the *Lycoperdaceae* and *Agaricaceae* families, but generally absent in log-growing mushrooms, suggesting the microbial community may influence As speciation in mushrooms. An As^{III}-S compound was identified in the XAS analysis, reportedly for the first time in mushrooms. The iAs predominated in most of the shop-bought mushrooms, albeit with low total As concentrations. In another study[36/04344], species transformation of As^{III} and As^{III} influx and efflux across the cell membranes of fungi *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and *Fusarium oxysporum* CZ-8F1 cells were studied using a cellular lysis plus HPLC-HG-AFS and XANES analysis. The results indicated that As^{III} can enter into fungal cells and that a portion of the As^{III} can be exuded out of cells. For both As sequestrated into fungal cytoplasm and As adsorbtion onto cell walls, As^{III} was found to be the dominated form of As. Among these fungal strains, however, there were obvious differences in the relative proportions of As^{V}, MMA, and DMA. The authors suggest that some of the intracellular As^{III} could be oxidated and methylated by these fungal strains and yield As^{V}, MMA, and DMA as product The transformations of As and potential formation of AB during the vegetative life stage (mycelium) of fungi has been investigated[36/05332].The mycelia of three different fungi species were cultured axenically and exposed to AB, As^{V} and DMA for 60 days. The mycelia of all fungi species accumulated all As compounds, although the accumulation of AB varied. Few bio-transformations were observed in these experiments indicating that it is unlikely that the mycelium of the fungus is responsible for biosynthesizing AB.

Arsenic in the marine environment continues to attract attention. Despite arsenoribosides and AB being the major As species in marine macro-algae and animals they have never been detected in seawater with only degradation products such as thio-arsenoribosides, dimethylarsinoylethanol (DMAE), DMA, MMA, and As^{V} having been reported. This question of the fate of organoarsenic species in marine ecosystems has been reviewed by Duncan et al., together with reaction kinetics of complex microbial communities[36/04759]. It was evident from this review that although liquid culture incubation studies are cheap and reproducible they lack the ability to culture representative microbial communities. Microcosm studies that include sand and sediment are more environmentally representative as they are a better simulation of marine ecosystems and are also likely to facilitate complex microbial communities. An added benefit of microcosm studies was that they are able to be run in parallel with field-based research to provide a holistic assessment of the degradation of organoarsenic species in marine environments. A second review by the same group focuses on if unicellular algae contribute to the formation of AB in higher marine organisms[36/02375]. Although arsenoribosides are the major As species in many marine unicellular algal species, AB has not been detected in unicellular algae which supports the hypothesis that AB is formed in marine animals via the ingestion and further metabolism of arsenoribosides. The observation of significant DMAE concentrations in some unicellular algal cultures suggests that unicellular algae-based detritus contains As species that can be further metabolized to form AB in higher marine organisms. The authors conclude that future research establishing how environmental variability influences the production of As species by marine unicellular algae and what effect this has on As cycling within marine food webs is essential to clarify the role of these organisms in marine As cycling.

The use of HPLC-MS for the simultaneous *determination of glycine betaine and AB in biological samples* has been described by Stiboller et al.[36/08710]. A water/methanol extraction was used followed by clean-up of extracts on a strong cation-exchange resin; the HPLC system consisted of a cation-exchange column with an ammonium formate buffer solution as mobile phase. Glycine betaine and AB were quantified in a single chromatographic run by splitting the HPLC flow with an adjustable flow splitter and detecting glycine betaine selectively by ESI-MS in the positive single ion monitoring mode at m/z 118, and AB with ICP-MS at m/z 75. The proposed method was validated for AB by analysis of CRM Dorm-2, and for glycine betaine by spiking the CRM Dorm-2 with a defined amount of glycine betaine. Finally, the developed method was applied to determine glycine betaine/AB ratios in single specimens of four species of marine fish and one species of freshwater fish. A study of lipid- and water-soluble As species in liver of Northeast Arctic cod (*Gadus morhua*) containing high levels of total As has also been published[36/05539]. The total As concentrations ranged from 2.1 to 240 mg kg^{-1} liver (ww) in the 26 samples studied. The As concentrations in the lipid fractions ranged from 1.8 to 16.4 mg As kg^{-1} in the liver oil, and a linear correlation was observed between the total As concentrations in liver and the total As concentrations in the respective lipid fractions of the same livers. The relative proportion of arsenolipids was considerably lower in liver samples with high total As levels (33-240 mg kg^{-1} ww), which contained from 3 to 7% of the total As in the lipid-soluble fraction. In contrast, liver samples with low As concentrations (2.1-33 mg kg^{-1}) contained up to 50% of the total As as lipid-soluble species. Arsenic speciation analysis of the lipid-soluble fractions of the livers, using reversed-phase HPLC-ICP-MS, revealed the presence

of several arsenolipids. Three major As-containing hydrocarbons ($C_{17}H_{39}AsO$, $C_{19}H_{41}AsO$ and $C_{23}H_{37}AsO$) and five arsenic-containing fatty acids ($C_{17}H_{35}AsO3$, $C_{19}H_{39}AsO_3$, $C_{19}H_{37}AsO_3$, $C_{23}H_{37}AsO_3$ and $C_{24}H_{37}AsO_3$) were identified using HPLC-qTOF-MS. Arsenobetaine was the major As species in the water-soluble fraction of the livers, while DMA, AC and iAs were minor constituents whilst iAs accounted for less than 0.1% of the total As in the liver samples.

Arsenic speciation studies in a range of terrestrial environments have been reported. A method has been developed for As speciation in anoxic pore waters[36/08256]. Using 25 µL aliquots, As^{III}, As^{V}, MMA^{V} and DMA^{V} were separated in less than 4 min by HPLC-ICP-MS using the IonPac^{R} AG7-AS7 anion-exchange column set and dilute HNO_3 as mobile phase. The LOD were below or equal to 0.25 µg L^{-1} for each As species.

In addition, no precipitation of iron and manganese (hydr)-oxides was observed since the anoxic samples were manipulated under a nitrogen atmosphere. Chlorides were eliminated by the chromatographic separation, thus enabling speciation analysis in estuarine or seawater samples. The method was validated using CRMs SLRS-4 and SLEW-3 and then used to determine iAs species in pore waters collected from the Marque River (Northern France) at concentrations below 1 and 10 µg L^{-1}, for As^{V} and As^{III} respectively. Others As species, identified as thioarsenic species, were also detected. The implications for As mobility in aquatic environments following the deposition of volcanic ash has been reported[36/01293]. Three samples of volcanic ashes collected after eruptions of the volcanos Hudson in 1991, Chaiten in 2008 and Puyehue in 2011 were analysed in order to define the solid speciation of As and the dynamics of its release to the aqueous phase. Bulk chemical analysis was carried out on the ground 63 µm fraction by ICP-OES after lithium metaborate/tetraborate fusion, and the composition of the near-surface region (2-10 nm) was determined XPS. Minerals present in the 63µm size-fraction of the samples were identified by XRD and SEM/EDXRD. The As compounds were found to be concentrated onto the ash surface in the form of As^{III}-S and As^{V}-O species. The As^{III} species were assigned to arsenian pyrite, while As^{V}-O compounds were assigned to adsorbed As^{III} ions or Fe arsenate salts precipitated as thin coatings. Although the main As carrier in the studied volcanic ashes is Al-silicate glass, this phase was stable at the neutral pH that dominates the aqueous reservoirs of the area affected by ashfall. Available As represented less than 6% of the total As measured. The effect of microbial SO_4^{2-} reduction and As mobility during flooding of contaminated soil has been studied[36/00111]. In the absence of significant levels of microbial SO_4^{2}- reduction, flooding caused increased Fe^{II} and As^{III} concentrations over a 10 week period, consistent with microbial Fe^{III} and As^{V} reduction. Microbial SO_4^{2-} reduction led to lower concentrations of porewater Fe^{II} as a result of FeS formation. SEM with EDXRF revealed that the newly formed FeS sequestered substantial amounts of As. Bulk and microfocused As K-edge XANES confirmed that As^{V} was reduced to As^{III} and showed that in the presence of FeS, solid-phase As was retained partly via the formation of an As_2S_3-like species. A tailings dam breach at an alumina plant in western Hungary in 2010 introduced $1x10^6$ m^3 of red mud suspension into the surrounding area. Red mud (fine fraction bauxite residue) has a characteristically alkaline pH and contains several potentially toxic elements, including As. A series of aerobic and anaerobic batch experiments have been made in order to investigate the *effects of red mud addition on soil biogeochemistry and As mobility*[36/00591]. Analysis of red mud by XAS showed that As was present as As^{V}. The

remobilisation of red mud associated As^{V} was highly pH dependent and the addition of phosphate to red mud suspensions greatly enhanced As release to solution. Dissolution of atmospheric CO_2 reduced pH, which resulted in a decrease of aqueous As concentrations over time. However, this did not result in complete removal of aqueous As in any of the experiments. The aqueous As concentrations initially increased in all experiments, and then remained relatively constant as the systems became more reducing. Both XANES and HPLC-ICP-MS showed that no As reduction processes occurred and that only As^{V} species were present. Anthropogenic As pollution in karst subterranean streams, based on a case study in Lihu, SW China has been reported[36/02553]. Analysis by both ICP-MS and XRF were used to elucidate As reaction mechanisms. The reducing environment in the subterranean stream resulted in As^{III} being the dominant species, accounting for 53% of the iAs. Most of the remainder was As^{V}, with very little MMA and DMA being detected. Following PCA of the influencing variables, the authors report that calcium and bicarbonate are the main influencing factors for water As adsorption in the study area. A very similar paper using slightly different software, has also appeared in a Chinese journal with different authors from the same institutes[36/01163]. In a study of natural systems, western clawed frog (*Silurana tropicalis*) embryos have been exposed to control, low (0.5 mg L^{-1}) and high (1 mg L^{-1}) As^{V} concentrations to investigate the effects As on different life stages[36/09765]. The effects were assessed by measuring As^{III} and DNA methyltransferases (AS3MT and DNMT1), as well as As speciation in the tissues. The As content in the frog tissues increased with water As concentration. Analysis using HPLC-ICP-MS revealed that the As was mostly iAs, DMA and TMAO, although using XANES, AB/TMA was also seen. Enzymes were also affected by the As exposure. Methylation of As to form MMA, DMA and TMAO in the frogs appeared to be inversely related to AS3MT levels. The authors suggest that a possible interpretation of this is that when AS3MT is higher, excretion of MMA + DMA + TMAO is more efficient, leaving lower concentrations in the tissues, with the opposite effect (less excretion) when AS3MT is lower; alternatively, alternative enzymes or linked genes may affect the methylation of As. A method has been developed for the determination of all five As species known to exist in atmospheric particulate matter (PM); i.e., As^{III}, As^{V}, MMA, DMA and TMAO[36/12021]. Although methylated species were first detected in PM in the late 1970s, most of the recent studies have focused mainly on the two iAs species, ignoring TMAO in particular. In this study, an HPLC (with an anion and cation exchange column connected in series)-HG-ICP-MS system provided complete separation of all five As species with LODs from 10 to 25 pg As mL^{-1}. The method was applied to water extracts of the inhalable fraction of atmospheric PM (PM10, PM2.5 and PM2.1). In total, 81 samples were collected, most during Saharan dust events, from a semirural area. The total water extractable As ranged from 0.03 to 0.7 ng of As m^{-3}, values that are representative for remote areas. The most abundant species was As^{V} followed by TMAO, DMA, As^{III} and MAA. None of the As species showed any particular trend with the presence or intensity of dust events, or seasonality, except for TMAO, which showed higher concentrations during the colder months. The determination of iAs species in ambient particulate matter (PM10 and PM2.5) have also been determined in the vicinity of a metallurgical industrial plant[36/11097]. The HPLC-ICP-MS method offered a LOQ of 0.34 ng m^{-3} for As^{III} and 0.23 ng m^{-3} for As^{V}. Good agreement was obtained between the sum of the concentrations of As^{III} and As^{V} and the total As content determined by XRF. Spiked As^{III} recoveries were 108% and 101% for PM10 and PM2.5 respectively. A significant conversion (oxidation) up to

54% of exogenous spiked As^{III} was observed. The average measured As concentration in PM10 during the 40-day monitoring campaign (30 ng m^{-3}) was above the European target value of 6 ng m^{-3}. The measured As concentration in PM2.5 was half the value of the measured concentration in PM10 and no relative enrichment of total As was observed in either particulate matter fractions. However, in PM10, As^{V} was the main component, while in PM2.5, As^{III} was the dominant species.

A number of groups have reported *clinical studies focusing on As speciation*. A study to elucidate the association of methyltransferase (AS3MT) haplotypes with As metabolism and the risk of basal cell carcinoma (BCC) has been reported[36/02391]. Four AS3MT polymorphisms were genotyped in BCC cases (N=529) and controls (N=533) from Eastern Europe with low to moderate As exposure (lifetime average drinking water concentration: 1.3 µg/L, range 0.01-167 µg/L). Urinary metabolites (iAs, MMA, DMA) were quantified by HPLC-ICP-MS. All haplotypes showed increased risk of BCC with increasing As exposure through drinking water. The results suggested that carriage of AS3MT haplotypes associated with less-efficient As methylation, or lack of AS3MT haplotypes associated with a more-efficient As methylation, results in higher risk of As-related BCC. The possible production of As haemoglobin adducts via exposure to arsine has also been studied[36/07091]. Preserved mouse blood was exposed to arsine in vitro, and the plasma separated. The residual clot of the control sample was then hemolysed using ultrapure water, and the supernatant collected. Plasma from mice exposed to arsine *in vivo* was also separated from blood. Immunoprecipitation assays were conducted after ultrafiltration, and three fractions were collected. The total As concentration in each fraction was quantified using ICP-MS. The three in vitro samples and the eluate fraction from immunoprecipitation were analysed with MALDI-TOF-MS. In the exposed samples, the As concentration in the fraction containing immunocomplexes was higher when immunoprecipitation was conducted with an anti-globin antibody. Three peaks were observed in arsine-exposed samples after MALDI-TOF-MS analysis. Two of them were around m/z 15,000, and the other was m/z 15,700. The latter peak was confirmed after immunoprecipitation. The authors concluded that the globin formed an adduct with As after both in vitro and in vivo exposure to arsine. This adduct together with haemoglobinuria could be a candidate biomarker of acute arsine poisoning in plasma. The International agency for research on cancer (IARC) has concluded that DMA^{V}, a metabolite of iAs, is responsible for carcinogenesis in urinary bladder and lung in rodents, and various modes of carcinogenic action have been proposed. However, the metabolic mechanisms of formation and disappearance of DMA^{III} and $DMTA^{V}$ (a metabolite of DMA^{V}) and their toxicity are not fully understood. A study to clarify these mechanism has been presented by Shimoda *et al*.[36/05568]. The *in vitro* transformation of arsenicals by treatment with liver homogenate from rodents and sulfur transferase was detected by HPLC-ICP-MS and HPLC-MS-MS. One species, $DMMTA^{V}$, was produced from DMA^{III} but not DMA^{V} by cellular fractions from mouse liver homogenates and by rhodanese from bovine liver in the presence of thiosulfate, a sulfur donor. The findings indicated that the metabolic process of DMA^{III} to DMA^{V} or $DMMTA^{V}$, and also of $DMMTA^{V}$ to DMA^{V} consisted of a complicated mode of interaction between monooxygenase including cytochrome P450 (CYP) and/or sulphur transferase. A sub-chronic study has been conducted to determine the As^{III} drinking water exposure dependent increases in female C3H mouse liver and lung tissue Asand to characterise the dose response (to 0, 0.05, 0.25, 1, 10, and 85 ppm As^{III} in drinking water for 30 days and a purified AIN-93M diet) for genomic mouse lung expression

patterns[36/11367]. Mouse lungs were analysed for iAs, MMA, and DMA by HG-AAS. The total lung mean As levels were 1.4, 22.5, 30.1, 50.9, 105.3, and 316.4 ng g^{-1} lung tissue after 0, 0.05, 0.25, 1, 10, and 85 ppm, respectively. At 85 ppm, the total mean lung As levels increased 14-fold and 131-fold when compared to either the lowest non-control dose (0.05 ppm) or the control dose, respectively. The As exposure elicited minimal numbers of differentially expressed genes (DEGs; 77, 38, 90, 87, and 87 DEGs) after 0.05, 0.25, 1, 10, and 85 ppm, respectively, which were associated with cardiovascular disease, development, differentiation, apoptosis, proliferation, and stress response. After 30 days of As^{III} exposure, the study showed monotonic increases in mouse lung As (total As and DMA) concentrations, but no clear dose-related increases in DEG numbers. The relationship between *As methylation and liver oxidative stress* induced by As_2O_3 has also been studied[36/09836]. Forty healthy mice were randomly divided into control group (0.9% saline) and As_2O_3 (1.0 mg/Kg/day, 2.0 mg/Kg/day, 4.0 mg/Kg/day) groups with gastric perfusion for five weeks. Arsenic species (As^V, As^{III}, MMA and DMA) were determined using HPLC-HG-AFS. The primary methyl index (PMI) and second methyl index (SMI) were calculated together with the level of hepatic function and activity which was detected with kits. It was found that the remaining As metabolic products in the liver significantly increased with the increasing doses of As_2O_3 and the liver function and oxidative stress deteriorated. The study demonstrated that the hepatotoxicity induced by the As accounted for deteriorating oxidative injury activised by As methylation metabolism, providing additional evidence to suggest a mechanism of As poisoning. A study to establish biomarkers of exposure to As on pregnant women and their children has been reported[36/05106]. The BEAR project was conducted in Gomez Palacio, Mexico based on 200 pregnant women. Concentrations of iAs in drinking water and maternal urinary concentrations of iAs, MMA and DMA were determined. Birth outcomes were analysed for their relationship to iAs in drinking water and to the concentrations and proportions of maternal urinary arsenicals. The iAs in drinking water ranged from < 0.5 to 236 µg As L^{-1} and more than half of the women (53%) consumed levels that exceeded the WHO recommended guideline of 10 µg As L^{-1}. Maternal urinary concentrations of MMA were negatively associated with new born birth weight and gestational age, and the maternal urinary concentrations of iAs were associated with lower mean gestational age and new born length. The biomonitoring results demonstrated that pregnant women in Gomez Palacio are exposed to potentially harmful levels of iAs in drinking water, with a clear relationship between iAs metabolism in pregnant women and adverse birth outcomes.

Table 2 shows selected examples of other applications of As speciation presented in the literature during the time period covered by this ASU.

Table 2 Applications of Speciation Analysis: Arsenic

Analyte species	Technique	Matrix	Sample treatment	Separation	LOD	Validation	Reference
AsV,	FAAS	Water and	Water: filtered,	UA-CPE of As^V 3-amino-7-	0.45 µg	NIST 1575a (pine	36/09

As^{III}		beverages	Chelex-100 column, evaporative concentration (x5); beverages: digested with nitric acid and hydrogen peroxide or perchloric acid; pine needles: no details	dimethylamino-2-methylphenazine, plus pyrogallol, extracted into surfactant-rich phase of polyethyleneglycolmono p-nonyphenylether. As^{III} oxidized with acid permanganate	L^{-1}	needles), NIST 1643e (trace elements in water) and spike recovery	326
As^{III}, As^{V}	HG-AAS	rice	50 g ground to <0.08 mm, (1) MAE (program given) of 500 mg with 5 mL 0.14 M nitric acid stand overnight, diluted to 14 mL, centrifuged 1600 rpm for 5 min. (2)	Selective HG from As^{III} with 0.1% (m/v) $NaBH_4$ in 10 M HCl. As^{V} reduced with ascorbic acid and KI in 1 M HCl	2 – 4 µg kg^{-1}	NIST 1568a (rice flour) ERM BC-211 (rice flour) and spike recovery	36/07 266

			heated (95 °C for 90 min) 250 mg with 5 mL 0.28 M nitric acid, centrifuged (no details)				
As^{III}, As^{V}	HG-AAS, Square wave cathodic stripping voltammetry	Lemongrass and turmeric	Freeze dried, UAE of 100 mg with 5 mL water for 90 min, centrifuged (50,000 g, 30 min).	As^{III} "soft generation condition, i.e., low HCl concentration." As^{V} reduction with ascorbic Only As^{III} was deposited on the Hg electrode when Cu was present in the HCl medium. As^{V} reduced with thiosulfate acid and KI	0.02 µg L^{-1} 0.5 µg L^{-1}	Institute of Nuclear chemistry and Technology Warsaw CTA-VTL-2 (Virginia tobacco leaves) and spike recovery	36/11 371
iAs and methylated As	HG-AAS	standards	none	Cryotrapped in liquid nitrogen, warmed up	Not measured	none	36/11 072
As^{III}, As^{V}	HG-AAS	blood	1 + 10 µL heparin, diluted to10 mL with DI-water,	DLLME of As^{III} as complex with tetraethylthiuram disulfide into mixed ionic liquids, 1-butyl-3-methylimidazolium hexafluorophosp	0.02 µg L^{-1}	NIST SRM 2670 (arsenic in freeze-dried urine)	36/05 573

				hate and 1-butyl-3-methylimidazolium tetrafluoroborate + Triton X-100 + acetone as dispersing solvent. Back extract into HCl. As^{V} reduced with KI and ascorbic acid			
As^{III}, As^{V}	ETAAS	Water	Filter (0.45 µm)	LLME of As^{V} isopolymolybdate with tetradecyl(trihexyl) phosphonium dicyanamide (ionic liquid) into tetrachloroethylene; As^{III} oxidised with peroxodisulfate at room temperature	0.002 µg L^{-1}	NIST 1643e (trace elements in water) and spike recovery	36/01 486
As^{III}, As^{V}	ETAAS	Rice, water	Rice: MAE dried, ground, 250 mg with 11 mL nitric acid and hydrogen peroxide (Program given). Diluted to	SPE As^{V} on 3-mercaptopropionic acid coated 3-aminopropyl triethoxysilane modified Fe_3O_4 nanoparticles, sonicated magnetic separation, dissolved in 1 M nitric acid. As^{III} oxidized with	0.010 µg L^{-1}	ERM-BC211 (rice), RTC-QCI-049 (water) and spike recovery	36/06 768

			200 mL. Water: acidifed to pH 2 with HCl, filtered (0.45 µm)	nitric acid and peroxide			
As^{III}, As^{V}	ETAAS	Rice	Ground to < 850 mm (sic), stored in desiccator for 1 week, UAE of 100 mg powder, 10 mL 0.5 M nitric acid, 136 min,	CPE of As^{V} isopolymolybdate in the surfactant-rich phase of Triton X-114. As^{III} oxidized with acid permanganate.	0.01 µg L^{-1} 0.033 µg L^{-1}	IRMM 804 (rice flour)	36/04 683
As^{III}, As^{V}	ETAAS	Rice	Grains rinsed, air-dried, milled to < 0.3 mm, MAE (program given) of 250 mg powder with 8 mL 1 M nitric acid, , centrifuged 6000 rpm for 10 min	DLLME of As^{III} diethyldithiophosphate. As^{V} reduced with thiosulfate and iodide	0.2 µg kg^{-1}	NIST 1568a (rice flour), NMIJ 7503a (white rice flour) and spike recovery	36/07 976
iAs	ET-AAS	water	UA extractio	SPE of As^{V} on 3-mercaptopropio	0.01 µg	RTC-QCI-	36/06

			n at pH 6 by 50 mg of modified nanoparticles, separated magnetically, UA elution with 1 mL 1 M nitric acid, separated magnetically	nic acid coated 3-aminopropyl triethoxysilane modified Fe_3O_4 magnetic nanoparticles. As^V oxidized with nitric acid and hydrogen peroxide.	L^{-1}	049 (water)	768
As^{III}, As^V	HG-AFS	Fruit juice	Filtered (0.45 µm)	DLLME of As^{III} with APDC into carbon tetrachloride, evaporated, residues dissolved in 1 mL 1 M hydrochloric acid, heated (65 ^oC for 15 min), iodide added. As^V reduced with thiosulfate. MMA interference removed by pH control of HG reaction.	1.2 µg L^{-1}	Spike recovery	36/11625
As^{III}, As^V	HG-AFS	Water	Acidified, filtered 0.45 µm)	CPE of (1) As^{III} with APDC, (2) As^V isopolymolybdate complex in	0.009 and 0.012 for As^{III} and	NIM GBW08605 (arsenic water)	36/10832

				Triton X-114. As^{III} extract diluted with hydrochloric acid (1 + 6), antifoam agent (400 µL) added. As^{V} extract diluted with hydrochloric acid and As^{V} reduced with thiourea-ascorbic acid.	AsV, respectively		
Inorganic arsenic	HG-AFS	rice	1000 mg ground powder (no details), 20 mL 0.02 M nitric acid, vortexed, heated (90 °C water bath 60 min), centrifuged (3300 g 10 min), filtered (0.22 µm)	Acidified to 10 M hydrochloric acid, As^{V} reduced with thiourea. As^{III} retained on Cleanert polystyrene SPE columns (60 mg, 3 mL), eluted with 2 mL water.	1.1 µg kg^{-1}	ERM BC211 (rice flour) NIST 1568b (rice flour) GBW 10043 (rice flour) and spike recoveries	36/11549
AsB, AsC, TMAO, MMA^{V}, DMA^{V}, As^{III}, As^{V}	ICP-MS, m/z 75, collision cell, H_2 + He (93 + 7).	oyster	10 g washed, freeze dried ground (< 1 mm), MAE (three	HPLC anion-exchange, PRP X-100, gradient (program given), ammonium carbonate, 20 min	Not given. 20 µg kg^{-1} estimated	NIST SRM 1566b (oyster tissue) and KRISS CRM 108-04-001	36/0669

			times, program given) of 100 mg with methanol : water (3 + 1), methanol removed (40 °C for 24 h), filtered (0.45 µm).			(oyster tissue)	
AsB, AsC, MMA^{V}, DMA^{V}, As^{III}, As^{V}	ICP-MS m/z 75, collision cell in kinetic energy discrimination mode, He.	Rice, rice products, apple and other juices	Rice and rice products: ground (cryogenic), 1000 mg extracted with 15 mL of 0.28 M nitric acid at 95 °C for 45 min, vortexed, and extracted for another 45 min, filtered (0.45 µm), diluted to 50 mL	HPLC ion-exchange, IonPac AS7, gradient (program given), ammonium carbonate, 15 min	Solution LOD of 0.004 µg L^{-1} for all species except As^{V} (0.07 µg L^{-1}). LOQ in samples of 2 – 10 µg kg^{-1}.	NIST 1568b (rice flour), NMIJ 7503a (white rice flour) and spike recovery.	36/01 413

			with 0.084 M nitric acid. Juices: 1000 mg diluted to 8 mL with water, mixed with 2 mL 0.32 M nitric acid, diluted as needed with 0.064 M nitric acid, filtered (0.45 µm).				
TeMA, AsB, AsC, TMAO, MMA^{V}, DMA^{V}, As^{III}, As^{V}	ICP-MS, m/z 75, collision cell, He	rice	500 mg extracted with 2 g of 0.15 M nitric acid at 100 oC for 2 h, 1 g of 50 µg kg^{-1} AsB solution and 7 g of water added, centrifuged (4000 rpm), filtered (0.45 µm)	HPLC reversed-phase, CAPCELL PAK C-18, Shim-pak VP-ODS C-18, Shim-pak VP-C8, DEVELOSIL C30-UG-5. 0.005 M ammonium dihydrogen phosphate in 0.05% acetonitrile, pH 2.7.	Not given	NIST 1568b (rice flour), NMIJ 7503a (white rice flour), ERM-BC211 (rice)	36/08 536

AsB, MMA^{V}, DMA^{V}, As^{III}, As^{V}	ICP-MS m/z 91 (AsO+), reaction cell O_2	honeysuckle	Dried, ground, MAE (program given) 500 mg with 5 mL 1% phosphoric acid, centrifuged (8000 rpm 5 min), repeated, combined, diluted to 20 mL, filtered (0.22 µm)	HPLC anion-exchange PRP X-1000, 0.008 M ammonium dihydrogen phosphate, pH 8	0.1 – 0.3 µg L^{-1}	GBW-10010 (rice flour) and spike recovery	36/11 993
MMA^{V}, DMA^{V}, As^{III}, As^{V}	ICP-MS m/z 81 (AsO+), DRC	Soil and plants	Soil: freeze dried, ground (150 µm), 200 mg extracted with 10 mL1 M phosphoric acid + 0.1 M ascorbic acid, centrifuged (8000g 10 min), filtered 0.22 mm (sic) Plants:	HPLC anion-exchange PRP X-100 0.020 M phosphate buffer, pH 6.2	0.3 – 0.7 µg L^{-1}	GBW07418 (soil), GBW07603 (aspen leaves)	36/01 080

			freeze dried, pulverized, as for soil				
AsB, AsC, TMAO, MMA^{V}, DMA^{V}, iAs	ICP-MS m/z 75	seafood	MAE 200 mg (lyophilized) or 1000 mg (fresh) with 10 mL 0.2% (w/v) nitric acid and 1 mL 1% hydrogen peroxide at 95 °C, centrifuged (3500 rpm for 25 min), filtered (0.45 µm)	HPLC anion-exchange (details in previous work) PRP X-100, cation-exchange Zorbax-SCX300, 0.02 M pyridine at pH 2.6	10 -28 µg kg^{-1} (dry weight)	NRCC DOLT-4 (dogfish liver), TORT-2 (lobster hepatopancreas) NIST 2976 (mussel tissue) and 1566b (oyster tissue) BCR-627 (tuna fish), ERM-BC211 (rice) ERM CE278 (mussel tissue) and spike recovery	36/03 387
AsB, AsC, MMA^{V}, DMA^{V}, As^{III}, As^{V}	ICP-MS m/z 75. Collision cell He	urine	1. Diluted 1 + 9 with 0.010 M potassium dihydrogen phosphate + 2% methanol + 20 µg L^{-}	HPLC anion-exchange PRP X-100, 0.005 M diammonium hydrogen phosphate + 0.005 M ammonium nitrate + 2% methanol + 20 µg L^{-1}	0.04 – 0.16 µg L^{-1}	NIST 2669 (arsenic species in human urine), comparison with another method (details not	36/06 859

			^{1} germanium at pH 5.8. 2. Diluted (1 + 19) 250 µL diluted with 250 µL 2% nitric acid and 4500 µL of 2% nitric acid + 0.2% sulfamic acid + 100 µg L^{-1} gold + 1.5% ethanol + 0.0005% Triton X-100 + 50 µg L^{-1} germanium + 50 µg L^{-1} rhodium + 60 µg L^{-1} rhenium	germanium, pH 9, 16 min		given)	
MMA^{V}, DMA^{V}, As^{III}, As^{V}	ICP-MS	groundwater	None described	CZE 60 cm,75 µm i.d., 375 µm o.d., fused silica capillary, 14 kV, 0.040 M boric acid +0.010 M sodium tetraborate buffer at pH 9.1.	0.2 – 0.5 µg L^{-1}	Spike recovery	36/00412

				Microconcentric nebulizer			
AsB, MMA^{V}, DMA^{V}, As^{III}, As^{V}	ICP-MS	River water	Acidified, filtered (0.22 µm)	HPLC anion-exchange PRP X-100 (10 cm), gradient, A: 0.020M NH_4NO_3 pH 8.7: B: 0.060 M NH_4NO_3 pH 8.7	0.01 – 0.2 µg L^{-1}	Spike recovery, no details given	36/09 693
As^{III}	TXRF spectrometry (portable S2 PICOFOX instrument)	water	pH adjusted to 5 with 0.1 M HNO_3 and 0.1 M NH_3	SPE of As^{III} on graphene oxide modified with 3 mercaptopropyl-trimethoxysilane. 200 µ L of GO-SH suspension (5 g L^{-1}) injected into 75 mL of sample, filtered, 0.5 mL of 2 mg L^{-1} Y (internal standard) in 2 M nitric acid added, sonicated (2 min), 10 µL suspension transferred to a siliconized quartz reflector and dried. As^{V} reduced with L-cysteine	0.064 µg L-1	LGC6016 (high salinity water– estuarine water from a heavily industrialized area) and BCR-610 (ground water).	36/05 582

4.3 Chromium

Efforts to develop new procedures for the speciation of Cr continue, apparently, unabated. Most of the applications are still for the analysis of environmental waters and other aqueous samples, but a number of applications to solid samples have been described in the current review period. Methods

based on selective extraction of species are, strictly speaking, fractionation methods even if speciation analysis has been performed on the extracts.

Two *reviews of Cr speciation* have been published. Markiewicz et al.[36/03923] emphasized metrological traceability in reviewing the literature, since 2000, concerned with the analysis of waters by HPLC-ICP-MS. The review contains a useful overview of the environmental aqueous chemistry of Cr^{III} and Cr^{VI} and a table in which the contents of 20 relevant articles are examined in some detail. The reviewers pay particular attention to the problems of validation, pointing out the difficulty of preserving Cr^{VI} species in CRMs and that spike recovery is not a "fully metrologically accepted" method of establishing traceability. They argue that species specific isotope dilution analysis (ssIDA) does provide the highest quality of metrological traceability and examine in some detail three papers that feature this method. Although they do not go so far as to argue that ssIDMS with HPLC-ICP-MS is the only possible way forward for the field of Cr speciation analysis, they are strongly supportive of the idea that laboratories working in this area invest in the technology and employ the highest standards of metrology that are now available. The review is base on 93 references (no titles). Jablonska-Czapla[36/04973] has, in addition to Cr speciation, reviewed the literature concerned with the speciation of As, Sb, and Tl by LC-ICP-MS in both waters and sediment samples (104 references with titles). The section devoted to sediment samples is based on only 12 references for all four elements, indicative of the relatively limited work on this topic; the bulk of the review is concerned with applications to water samples.

Several *applications of X-ray spectroscopies* for Cr speciation studies have been described. Full spectral XANES imaging was applied to the direct speciation analysis of chrome yellow pigments in paintings by Vincent van Gogh[36/05290] to study the photo-reduction of chromates to Cr^{III} compounds that is the cause of darkening these pigments. The researchers have overcome the limitations of instrumentation used in previous studies in terms of limited count rate capabilities and collection solid angle by the development of the 384 silicon-diode array Maia XRF detector, which allowed for full spectral XANES imaging in XRF mode so that spectroscopic information was available at each pixel in the scanned map. The results collected from two micro-samples from two Van Gogh paintings and an aged paint model showed the feasibility of performing full spectral XANES imaging in realistic times (from several minutes to a few hours) and also identified $Cr(OH)_3$, Cr^{III} sulfates and/or Cr^{III} organometallic compounds at the microscale (less than 1 mm) over the entire scanned area. Delay et al.[36/08153] developed a speciation method for solid waste materials by X-ray absorption spectroscopy and, for the species in the leachates, a method based on IC-ICP-OES. They also applied XAS to the liquid samples as a way of validating the IC-ICP-OES results. Measurements were made at XAS beamline at the Angstrom Light Source, Karlsruhe. Liquid samples were injected into a cell made by welding together, polyethylene terephthalate, polyethylene (PE) and ethylene vinyl alcohol foil cut accordingly to give a cell of dimensions 20 mm x 25 mm x 1 mm). Not only could both species be detected at concentrations below 1 mg/L, it was also possible to follow the conversion of Cr^{VI} to Cr^{III} and this process was studied as a function of the pH. Although the authors do not advocate XAS as a routine method for Cr speciation in the liquid phase, they do point out that the further development of silicon drift detectors will decrease the quantification limit and that the six-element 100 mm^2 detector (currently in commissioning at ANKA) will decrease

the limit by at least a factor of 5. In another study, researchers extracted Cr^{VI} from drinking waters by dispersive micro solid-phase extraction onto multiwalled carbon nanotubes, modified with the anionic exchanger tricaprylmethylammonium chloride (Aliquat 336), followed by quantification by TXRF spectrometry[36/06020]. After sorption, the aqueous sample was separated by centrifugation and the loaded MWCNTs were suspended using a small volume of an internal standard solution and analyzed directly by a benchtop TXRF spectrometer, without any elution step. Concentrations down to 3 μg/Lcould be detected, well below the WHO maximum permissible limit. The procedure was validated by spike recoveries, at concentrations up to 50 μg L^{-1}, from mineral and tap waters, whose concentrations were below the LOD of the method.

Several methods based on *preconcentration by selective extraction with quantification by AAS* have been developed. Diniz and Tarley[36/10627] devised a FAAS procedure in which Cr^{VI} was retained at pH 5.0 onto mesoporous amino-functionalized Fe_3O_4/SiO_2 nanoparticles followed by elution with 0.5 mL of 2.5 M HCl. The Cr^{III} remaining in the solution was concentrated by CPE after reaction with 4-(2-thiazolylazo)resorcinol (TAR) in 0.3% (v/v) Triton X-114. The preconcentration factors were 16 and 12, and the LOD were 1 and 3 μg/L for Cr^{VI} and Cr^{III}, respectively. The procedure was validated by (a) spike recoveries, at concentrations of 45 μg/L, from mineral, lake and tap waters, whose Cr^{VI} concentrations were not detectable, and (b) the determination of total Cr as Cr^{VI} following an oxidative sample dissolution of a fish CRM (DORM-2 fish protein). Other researchers[36/07781]developed a procedure in which only the Cr^{VI} was preconcentrated, this time on amino bimodal mesoporous silica nanoparticles, packed into a small column. The pH of the sample (100 mL) was first adjusted to 2 with nitric acid, and after washing the column, the Cr^{VI} was eluted with 1.5 mL of 0.3 M sodium hydroxide solution and measured by FAAS. Total Cr was determined after oxidation with peroxide, and Cr^{III} was determined by difference. The LOD was 1 μg/L and the method was validated by spike recoveries (up to 50 μg/L) from several real samples, almost all of which contained measurable concentrations of both species and by the accurate analysis of NIST SRM 1643e (Trace elements in water), which contains Cr at a concentration of 20 μg/L. A procedure in which the two species are separated by *in situ* DLLME based on the formation of ionic liquids has been devised[36/10117]. Two versions of one half of the molten salt were synthesized by derivatizing 1-bromoethyl-3-methylimidazolium bromide with 8-hydroxyquinoline (8-HQ), for the retention of Cr^{III}, and with ammonium pyrrolidine dithiocarbamate (APDC), for the retention of Cr^{VI}. The Cr^{III} version was first added to the sample, together with Triton X-114, $NaNO_3$, and an acetate buffer (pH 5, 0.05 M) followed by the other half of the molten salt and KPF_6. The droplets of ionic liquid were settled by centrifugation, removed and diluted with ethanol prior to analysis by FAAS. Then, the other derivative was added and the procedure repeated to collect the Cr^{VI}, though this time the ionic liquid collected by centrifugation was diluted with isopropanol before measurement by FAAS. The reported LOD values were 6 and 11 μg/L for Cr^{III} and Cr^{VI}, respectively. The method was validated by spike recoveries, at concentrations up to 400 μg/L, from several waters and a urine, none of which contained measureable concentrations of either Cr species. An SPE-FAAS method with an LOD of 3 ng/L has been reported[36/01888], but it turns out that this is only achieved for the passage of 1000 mL through the column, which at a flow rate of 2 mL/min would take in excess of 8 hours. The method is based on the selective retention of Cr^{VI}, from solutions at pH 3, on the chloride form of Lewatit Ionac SR-7 ion-exchange resin followed by elution

with 3 M nitric acid. Total Cr was determined after oxidation with peroxide, and Cr^{III} determined by difference. The method was validated by the accurate analysis of CRMs (SPS-WW2 Batch 108 wastewater, and BCR 701 lake sediment). Spikes at concentrations of 5 mg/L were recovered from several samples and the method was applied to a number of waste waters all of which contained measurable concentrations of both species ranging from 32 µg/L to 63 mg/L.

Lower Cr concentrations can be measured when the *extraction is combined with ETAAS*. A calibration range of 5-100 ng L^{-1} was obtained in a CPE procedure in which the sample was acidified (pH 2) and heated to 60 °C before the addition of silver nanoparticles and Triton X-114[36/03899]. According to the researchers, total Cr was transferred to the surfactant-rich phase, 10 µL of which was transferred to the electrothermal atomizer. When the procedure was repeated with the addition of EDTA immediately before the addition of the nanoparticles, the signal corresponded to the concentration of Cr^{VI} in the sample. The researchers consider that as only Cr^{III} is extracted by the CPE procedure, the Cr^{VI} was first reduced by the EDTA, but reacted with the nanoparticles rather than the excess EDTA, as this latter reaction is slow. The reason why total Cr is determined in the absence of EDTA was not explained. The method was validated by the accurate analysis of three CRM (NIST SRM1640a, natural water, NRCC NASS-6, seawater, and SPS-SW2, surface water), and by spike recoveries of both species at concentrations up to 50 ng/L, from several water, wine and beer samples, all of which (with the exception of some beers) contained measureable concentrations of Cr. The enrichment factor was 1150 and the LOD was 2 ng/L. Another way to achieve such high preconcetration factors is to extract into a microdroplet. A procedure based on this approach[36/01084] has been devised in which the Cr^{III} was selectively extracted at pH 6 as the complex with 8-hydroxyquinoline into 1-decanol. The sample (30 mL) was buffered at pH 6 and heated to 45 °C in a 50 mL screw cap vial containing a magnetic stir bar that was rotated at 450 rpm to produce a stable vortex at the surface, into which was deposited 130 µL of decanol. After 50 min, the decanol droplet was removed with a micropipette, diluted to 600 µL with methanol containing 0.1 M nitric acid, and a 20 µL subsample injected into the electrothermal atomizer. Total Cr was determined after reduction with hydroxylamine hydrochloride. The LOD was 30 ng/L. The method was validated by the analysis of two environmental water CRMs (GSBZ50027-94 and GBW(E) 080642, Institute for Environmental Reference Materials of Ministry of Environmental Protection, Beijing, China), and spike recoveries of both species at 0.4 µg/L from a well and a tap water sample, neither of which contained measureable concentrations of Cr^{VI}.

Two procedures based on *determination by atomic emission spectrometry* have been developed. In the first[36/00631] the Cr species were extracted from sediment samples (0.1 g) into a solution made up of 3 mL of 0.1 M EDTA, 0.5 mL of 1% tetrabutylammonium bromide and 0.5 mL of HF in a 15 mL polyethylene centrifuge tube, which was irradiated at maximum power (700 W) in a domestic microwave oven. The anionic Cr^{VI} was retained on the sulfate form of Dowex-1 at pH 4.5, whereas the EDTA complex of Cr^{III}, although also anionic, was not retained. The adsorbed Cr^{VI} was eluted with 2 M nitric acid, and the solutions analyzed by ICP-OES. Total Cr in the extract was also measured. The procedure was validated by the analysis of a stream sediment CRM (GBW-07312, certified only for total Cr), and by spike recoveries (concentrations not given) of both species from two sediment and two soil samples, one each of which did not contain measureable amounts of

Cr^{VI}. The solution LOD was 20 µg/L which corresponded to 5 mg/kg in the solid samples. A similar principle was applied in the second procedure for the analysis of waters by solution-cathode glow discharge-atomic emission spectrometry[36/00611]. The Cr^{VI} was selectively retained on a column of lysine-modified mesoporous silica (Fmoc-SBA-15) at pH 5 in a flow injection system. In a second step Cr^{III} was oxidized to Cr^{VI} with peroxide. The Cr^{VI} was eluted with 0.1 mol/L ammonia solution. The enrichment factor was 91, leading to an LOD of 0.8 µg/L for Cr^{VI}. The method was validated by the accurate analysis of a riverine water CRM (GBW08607) and by spike recoveries at concentrations up to 100 µg/L from two water samples, neither of which contained measurable amounts of either species.

Several procedures based on *separation with detection by ICP-MS* have been reported, most of which feature an high performance separation coupled directly with the spectrometer. For the analysis of drinking and tap water, Cr species were separated by HPLC on a reversed-phase C8 column by isocratic elution and a mobile phase of 5 mM EDTA at pH 7 and 40 °C which ensured the rapid formation of the CrEDTA complex[36/11340]. Potential interferences at m/z 52, e.g. from $^{40}Ar^{12}C^{+}$ were removed by reaction with ammonia in a dynamic reaction cell. The researchers present results that show a possible conversion of Cr^{VI} to Cr^{III} when the solutions containing the EDTA were incubated for two hours. The method was validated by analysis of one CRM (NIST SRM 1643e) and by spike recovery at concentrations up to 5 µg/L of both species from this material, which contains only Cr^{III}. The method was applied to several tap waters, most of which contained measureable concentrations of both species, and to some mineral waters, about half of which also contained measureable concentrations of either species. The LOD was 0.1 µg/L. A procedure for the determination of not only Cr species, but also those of As (inorganic arsenic, MMA and DMA) and Cd^{II} with ion-exchange (Hamilton PRP X-100 column) HPLC has been devised[36/05633]. Anionic complexes of Cr^{III} and Cd^{II} were formed by the addition of EDTA and the mobile phase was 40 mM ammonium nitrate at pH 8.6. The LOD values ranged from 0.07-0.12 µg/L, and for the Cr species were 0.1 and 0.7 µg/L for Cr^{III} and Cr^{VI}, respectively. The method was applied to the determination of the target species in several surface water and drinking water samples, all of which contained measurable concentrations of all species except MMA and DMA. Spike recoveries from these samples ranged from 90 to 116%. A method for the determination of Cr picolinate, Cr^{III} and Cr^{VI} in foods, based on a CZE separation has been devised[36/07276]. Following UAE, Cr^{III} was chelated with trans-1,2-diaminocyclohexane-N,N,N',N '-tetraacetic acid (45 min at 60 °C) to form a singly charged complex, and all three species were separated by CZE within 8 min at a separation voltage of -13 KV (i.e. in reverse CE mode) in a running buffer solution consisting of 25 mmol/L NaH_2PO_4, 6.25 mmol/l $Na_2B_4O_7$, and 0.6 mmol/L CTAB at pH 7.80. The separation system was connected to an ICP-MS instrument (fitted with a microconcentric nebulizer) by the CEi-SP20 CE-Interface system (Reeko Instrument, Xiamen, China). Signal intensity at m/z 52 was monitored and the dynamic reaction cell contained hydrogen as the reagent gas. The procedure was applied to the analysis of one Cr supplement for which the UAE conditions were specifically optimized in terms of solvent (water:methanol 25:75), time (20 min) and solvent volume in mL to sample mass in g (40). The sample contained only Cr picolinate (at a notional Cr concentration of 1500 mg/kg). Spike recoveries of all three species, added at concentrations of between 500 and 1000 mg/kg, were between 93 and 103%. The reported solution LOD values were 0.10, 0.18, and 0.20 µg/L for Cr^{VI}, Cr^{III}, and Cr

picolinate, respectively. As the authors indicated that 0.1 g of supplement was dissolved in 10 mL of buffer, there must have been a concentration step in the procedure that was not described in the paper. In another study of foods, the interaction of Cr^{VI} was determined by SEC, while the stability was followed by ion-exchange HPLC[36/06991]. Total Cr was also determined after digestion with nitric acid and hydrogen peroxide. The instrument was operated without the use of a collision/reaction cell and so the chromatograms contained a large early peak mostly due to polyatomic interferences. For the SEC (Superdex-200 HR 300 mm x 10 mm column), the mobile phase was 30 mM Tris buffer at pH 7.5 flowing at 0.7 mL/min for 45 min. The volume injected was 100 µL, and the signals at m/z 52 and 53 were monitored. For the ion-exchange HPLC (CS5A 250 mm x 4 mm i.d. column), the mobile phase was 0.085 M HNO_3 at 1 mL/min with an volume injected of 100 µL. After separation, a 5-min cleaning step with 0.35 M HNO_3 was applied. Samples were extracted with ammonium hydroxide at pH 11.5 and centrifuged and ultrafiltered as needed. The methods were validated by the analysis of several CRM: lobster hepatopancreas (TORT-2, NRC), a bovine muscle (SRM 8414, NIST), and a whole egg powder (SRM 8415, NIST), for which only an indicative value is given. The LOD values ranged from 1 to 10 µg/kg depending on the matrix. The method was applied to the determination of Cr^{VI} in several foodstuffs (dairy products, flour, chocolate, vegetables, fruits, meat, fish, eggs, and beverages) from different brands and origins, none of which contained measurable concentrations of Cr^{VI}. The stability of Cr^{VI} spiked into a semi-skimmed cow milk sample was studied, and it was found, not surprisingly, that the degradation rate, which was substantially greater than that in pure water, increased with temperature. Scancar et al. have extended a previously developed HPLC separation for chromate, to the determination of molybdate, tungstate and vanadate in the alkaline extracts of welding fume[36/08654]. A strong anion-exchange fast protein liquid chromatography polystyrene/ divenylbenzene column (Mono QHR5/5, Pharmacia) matrix, was used. The outlet of the chromatographic column was directly connected to the Miramist nebuliser of the ICP-MS instrument. An internal standard of 100 µg/L germanium, scandium, rhodium and bismuth was added to the eluate post-column. To minimise polyatomic inferences at m/z 50 and 52 a high energy collision mode was applied with helium as the collision gas. Analyses in carbonate buffer matrices with NaCl (4%) as eluent were possible, though for the chromatographic separation a linear gradient elution from 100% water to 100% 0.7 M NaCl was applied. The procedure was applied to the determination of leachable concentrations of chromate, molybdate, tungstate and vanadate in alkaline extracts (2% NaOH and 3% Na_2CO_3) of metal arc welding fumes loaded on filters. The LOD values were 0.02, 0.1, 0.1 and 0.2 µg/L, for Cr, Mo, V and W, respectively. The method was validated by accurate analysis of BCR CRM 545, (Cr^{VI} in welding dust loaded on a filter) and by spike recoveries of 10 µg/L spiked into the alkaline extract of one sample.

The creation of a *Cr^{III}-selective solid phase extractant by an evolutionary approach based on phage display peptide library* has been demonstrated[36/12109]. The method has been applied in the past to generate peptides selective towards metals such as Cd, Ni, and Pb, but has not been used to create extractant materials. The authors consider that a phage bearing a metal-binding peptide is an ideal SPE sorbent, as the dense proteinaceous cylinder coat makes the M13 phages both thermally and chemically stable. The small size (6.5 nm x 900 nm) and multiplicity of metal binding sites also endows it with higher specific surface area in comparison with other microbial cells. The

selection procedure, known as “biopanning,” is described in detail in the paper, and phages bearing the peptide YKASLIT were finally chosen and immobilized on porous spherical cellulose particles of diameter 200 – 280 µm. The sample (adjusted to pH 7) volume was 4 mL and the eluent (0.1 M nitric acid) volume was 400 µL, giving an ICP-MS LOD of 20 ng/L. The material also reacts with Cr^{VI}, which was removed with native Cytopore which is selective for Cr^{VI} at pH 7. The procedure was validated by the analysis of a CRM (GBW08608 simulated water) and by recoveries at concentrations of 0.1 µg/L from a snow-melt and a tap water, both of which contained measurable concentrations of both species.

4.4 Gadolinium

One paper on the application *of gadolinium (Gd)-based contrast agents for magnetic resonance imaging (MRI) in water* has been published during the period covered by this review. The method employs a new zwitterionic HILIC (250 mm × 2.1 mm × 3 µm) column coupled to ICP-MS to determine gadolinium compounds in tap water[36/0518]. This methodology allowed the authors to obtain LOQ values between 5- 12 ng/L for five Gd-based contrast agents: gadobenate: (Gd-BOPTA), gadodiamide (Gd-DPTA-BMA), gadobutrol (Gd-BT-DO3A), gadoterate (Gd-DOTA) and gadopentetate (Gd-DTPA). Additionally, the use of as internal standard, Pr-DOTA (a metal macrocyclic chelate produced by chelating of the Pr (III) ion with DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)) notably improved the RSD values from 4% to 1.8% without and with adding the internal standard, respectively. By applying these conditions GdBOPTA, Gd-BT-DO3A and Gd-DOTA were identified and quantified in tap water samples collected in two suburbs in Berlin at levels ranged from 10 to 20 ng Gd/ L. Separation was accomplished in less than 30minutes.

4.5 Iron

4.6 Halogens

In response to the inclusion of polybrominated diphenyl ethers (PBDEs) in the list of priority substances in the European Water Framework Directive (WFD) the group of Prange at the Institute of Coastal Research in Germany, have developed a GC-ICP-MS method[36/01474] for the *high accuracy measurement of PBDEs in surface waters at low concentrations by detection of Br* and then compared it to other routine GC-based methods[36/11497]. For PBDEs the WFD specifies Environmental Quality Standards (EQS) at trace concentrations corresponding to 0.5 ng L^{-1} for 6 priority brominated flame retardants. Within this context a GC-ICP-MS method was developed and optimized allowing the ultra-trace determination of the priority congeners at sub ng L^{-1} levels. The method used cool on column injection onto a DB5MS column (15 m x 0.25 mm i.d., film 0.1 □m) and a temperature controlled Silcosteel® transfer line with a heated stainless steel injector tip to

interface the GC and ICP-MS systems. The dry plasma conditions were optimised for the detection of Br using a spiked water sample which was extracted using a hexane:DCM (1:1) mixture, which was subsequently reduced to 50 - 75 ?L. A low power plasma (750 - 850 W) was optimal and ionisation of the high 1st ionisation potential Br atoms was enhanced by addition of 40 mL min^{-1} He to the plasma. The quantification approach was based on the application of ^{81}Br labelled PBDEs as internal standards for ssIDMS and samples of natural water were analysed to demonstrate the potential of this new detection and quantification method. The main drawback of the approach was the potential lack of selectivity in the presence of other Br-containing organic compounds which could co-elute with the compounds of interest. Further work on the sample preparation methods to remove any interferences was proposed. In a second paper[36/11497], the instrumental capabilities of the MS techniques most frequently used in the determination of PBDEs, namely GC-negative-chemical-ionisation-MS (GC-NCI-MS) and GC-electron-ionisation tandem MS (EI MS/MS), were evaluated in comparison with the developed GC-ICP-MS method for the reliable determination of PBDEs. Three analytical methods based on the liquid-liquid extraction of water samples and measurement of the extracts by GC-NCI-MS, GC-EI MS/MS, or GC-ICP-MS are described. The priority PBDEs were quantified in different types of water sample by means of IDMS using ^{81}Br-labelled or ^{13}C-labelled PBDEs spikes, depending on the selected ionisation source. The three proposed methods met the requirements of the European legislation in terms of LOQs and expanded uncertainties. The determination method using ^{81}Br-labelled PBDEs and GC-ICP-MS had the highest sensitivity and the lowest instrumental LODs and expanded uncertainties.

The development of a GC-ICP-MS/MS method[36/07656] for the *selective and sensitive detection of heteroatom containing compounds via P, S and Cl* has been reported. As a proof of concept, organophosphorus, organosulfur, and organochlorine pesticides in various food matrices were measured. For the multi-heteroatom detection of organophosphorus and organosulfur pesticides, oxygen was used in the collision reaction cell (CRC) to convert P (m/z 31) to PO^+ (m/z 47) and S (m/z 32) to SO^+ (m/z 48). In a separate run, ClH_2^+ (m/z 37) was monitored after the reaction of Cl (m/z 35) with hydrogen in the CRC for the determination of organochlorine pesticides. In ICP-MS/MS the first quadrupole (Q1) acts as a mass filter and in the CRC that follows, analytes or interferences are reacted with various gases in a very selective way to produce a product ion, which is then accepted (analyte) or rejected (interference) by the second quadrupole (Q2) prior to detection. Using this approach, background levels are dramatically reduced, effectively increasing the sensitivity of detection. In the current application the instrumental parameter settings were optimised manually using Ar gas spiked with 100 mg L^{-1} of H_2S, unfortunately this was problematic as the S contaminated the flow system and continued to bleed out for the duration of the study. A better choice would be to introduce the H_2S/argon mixture as close to the ICP torch as possible, thereby minimizing the contact area for S adsorption. Real food samples including baby food purees, fresh vegetables, loose tea, were screened for their pesticide content, following preparation of triplicate extracts using a QuEChERS approach (quick, easy, cheap, effective, rugged, and safe). Excellent linearity with correlation coefficients greater than 0.997 were achieved and the LODs obtained for the organophosphorus, organosulfur, and organochlorine pesticides were 0.0005, 0.675, and 0.144 ?g kg^{-1}, respectively.

A rapid method for the *speciation of iodine in human serum and urine by HPLC-ICP-MS* has been reported[36/01749]. The separation used an anion exchange column (AG 11, Dionex) with gradient elution involving a pH 8 Tris-ammonium acetate buffer with methanol elution and detection of ^{127}I by ICP-MS. Despite a clear baseline shift during elution of iodide this could be corrected and separation of iodide from other iodine containing species including: di-iodothyronein; thyroxin (T4); and tri-iodothyronine (T3), was possible in 8 min. The method was validated in human plasma and urine using Seronorm ClinChek plasma and urine IQC materials, providing good recovery and precision and generating an LOD for iodine of 0.12 □g L^{-1} and 0.05 □g L^{-1} in serum and urine respectively. The method was applied to a small number of human serum and urine samples and provided acceptable results.

Methods[36/11577][36/11333] for the *analysis of simple iodine containing species in tissue samples using IC coupled to ICP-MS detection* have been developed. Investigations into the fortification of food with I containing compounds has resulted in the requirement to measure iodate (KIO_3) and iodide (KI) which are the main salt iodization agents used worldwide. In the first study[36/11577], a method for the measurement of IO_3^- and I^- in tissue homogenates using HPLC-ICP-MS was developed and used to demonstrate the KIO_3 reduction process by tissues *in vitro*. A protocol[36/11333] allowing the determination of the total I content and I containing species in samples of animal tissues using ICP-MS as an element-specific detector has also been developed. The total I content was determined after microwave digestion with TMAH (25% w/w). The detection limit was 26.9 g kg^{-1} and the accuracy of the determination was demonstrated by the analysis of three SRMs: non-fat milk powder; porcine liver; and Atlantic cod muscle samples, using calibration by standard additions. The extracts for the speciation analysis were prepared through sample dispersion in water using an Ultra-Turrax system. The extraction yields ranged from 46 to 84% for different types of tissues. The determination of the inorganic I containing species was performed using AEC-ICP-MS, with a PRP X100 column and an eluent containing 100 mM ammonium nitrate at pH 7.4. A detection limit of 1.1 g kg^{-1} as I was obtained for both species. The organic I containing species were separated using SEC-ICP-MS using a Superdex 75 column and mobile phase containing 20 mM Tris-HCl at pH 7.5. Samples of porcine muscle, liver, kidney and thyroid gland, chicken muscle and liver and Atlantic cod muscle were analyzed. The porcine thyroid gland and Atlantic cod muscle samples contained more than 10x greater content of I than the other samples. With respect to the inorganic species, only iodide was found in the sample extracts. Conversely, many organic iodine species were found in the extracts.

A method based on the use of GC-ICP-MS/MS is claimed to be the first presented for the detection of specific heteroatoms of P, S and Cl, and the subsequent application to the determination of organophosphorous, organosulfur and organochlorine pesticides in food matrices. To detect organophosphorus and organosulfur pesticides, oxygen was used in the collision reaction cell to convert P (m/z 31) to PO^+ (m/z 47) and S (m/z 32) to SO^+ (m/z 48) while hydrogen was employed to transform Cl (m/z 35) to ClH_2^+ (m/z 37) for the determination of organochlorine pesticides. Different food matrices were analyzed: baby food fruit purees, green onions, tea (three types), green peppers, and yellow onion. To extract pesticides from food matrices a QuEChERS method was applied. The LOD values achieved were 0.0005, 0.675 and 0.144 µg/kg for organophosphorus, organosulfur, and

organochlorine pesticides, respectively. Compared to GC-MS-MS the LOD obtained for organophosphorus pesticides was improved by one order of magnitude whereas for organosulfur, and organochlorine pesticides both GC-MS-MS and GC-ICP-MS-MS provided comparable LOD values.

4.7 Lead

A *fast HPLC-ICP-MS method has been described for lead speciation* in water samples[36/10969]. Separation of Pb^{II}, TMPb, TEtPb and TPhPb was achieved in less than 3.5 minutes by using a C_{18} (4.6 mm x15 cm x 5 mm) column. The mobile phase consisted of 5 mg/L sodium 1-pentanesulfonate at a pH of 5, and a gradient elution (5–90 percent methanol in 1 minute) flowing at 1.2 ml/min was used. The reported LOD values were 0.01 and 0.02 mg/L for Pb^{II} and organic lead, respectively which were low enough for routine lead speciation in environmental water without the need of a pre-concentration step. The recovery rates and RSDs were higher than 93% and lower than 3% respectively. Method validation was undertaken using two CRMs (Water (GSBZ 50009-88, Beijing, China) and seawater (GBW (E) 080040, Hangzhou, China) with results in good agreement with the certified values.

4.8 Manganese

Exposure to Mn either environmentally or more usually occupationally can lead to an increasing concentration of this element in the brain. The consequence of this is an irreversible damage of doparninergic neurons leading to a disease called manganism with a clinical presentation similar to the one observed in Parkinson's disease. The group of Michalke has worked for a number of years to elucidate the toxicological mechanism behind these affects. In two recent studies[36/09996][36/11754], they have investigated the pattern of the Mn-species in serum by SEC-ICP-MS and then assessed the developed biomarkers in human populations from Munich, the Emilia Romagna region in Italy and from Sweden. For the optimum separation of Mn species in serum, a combination of two SEC columns were used, equipped with a precolumn to retain contaminants. The first column with a PEEK body (250 × 8 mm) was packed with Toyopearl HW55SF for the separation of high molecular mass (HMM) compounds, while the second PEEK column (200 × 8 mm) was packed with Toyopearl HW40S for the separation of low molecular mass (LMM) molecules. This combination provided a satisfactory separation of LMM compounds like Mn-citrate from HMM compounds. The isocratic eluent was composed of 90% eluent A (50 mM ammonium acetate, pH 5.8) mixed with 10% eluent B (10 mM Tris, 50 mM ammonium acetate, 5% (v/v) MeOH, pH 8) and was delivered by a metal-free HPLC system with a flow rate of 0.7 mL min^{-1}. The results for Mn-speciation in two animal models, were correlated to the brain metabolome determined by means of ESI-ICR/FT-MS. The powerful combination of Mn speciation in serum with metabolomics of the brain underlined the need for Mn-speciation in exposure scenarios instead of the determination of total Mn concentration in blood. The developed biomarkers were then used to biomonitor human populations in three European countries. The authors showed that at a low total Mn concentration a change in the major Mn carrier in serum takes place from Mn-transferrin (Tf) towards Mn-citrate. A simpler method for the Mn-citrate determination, using ultrafiltration (UF) of serum samples was tested for suitability, the latter possibly being a preferred choice for routine

occupational medicine laboratories. The results indicated that UF could be an alternative if methodical prerequisites and limitations, such as using a thorough cleaning procedure, were followed.

4.9 Mercury

It is evident from *the papers covered in this year's ASU review*, and those in recent years, that Hg speciation analysis using alkylation and GC as the separation technique, coupled with either AFS or ICP-MS as the detector, can be considered as a mature field with few novel improvements reported. However, for separations involving HPLC the optimisation of various method parameters, including the choice of stationary phase, column dimensions and mobile phase composition and pH continue to be reported. Whether this is due to new workers entering the field and starting method development from scratch, to the nature of Hg speciation by HPLC, or arising from the use of the wide variety of HPLC columns available, or a combination of all three is difficult to discern. This suggests a need for a comprehensive critical review of the topic that gives details of columns and mobile phases used, species separated and retention times and method validation. It is also apparent, from the number of papers recently published on the topic, that IDA applied to Hg speciation studies, once the preserve of national measurement institutes, is now in widespread use in research laboratories worldwide. The next step will be for the wider community to fully embrace the concept of measurement uncertainty, by the use of full uncertainty budgets, for which the technique is ideally suited.

4.10 Molybdenum

Molybdenum precipitates are considered to be indicators of paleoredox conditions. A crucial step in the precipitation of molybdenum in anoxic environments is the formation of thiomolybdates ($MoO_{4-x}S_x^{2-}$ with x values of 1−4) as intermediates. A method has been developed to determine the concentration of thiomolybdates in natural waters using an ion pair chromatographic (Dionex, IonPac NS1, 250 × 4 mm x10 µm) column on line coupled to ICP-MS[36/05202]. The use of 2-propanol (10%-25% gradient) in the mobile phase (2mM tetrabutylammonium hydroxide/1mmol/L Na_2CO_3) was found to decrease the formation of carbon, thus eliminating the need of oxygen addition to the instrument plasma gases. Additionally, the column was heated to 30 ºC to decrease the viscosity of the mobile phase. Under these optimal conditions, five Mo species (molybdate, monothiomolybdate, dithiomolybdate, trithiomolybdate and tetrathyomolybdate) were baseline separated in less than 15 minutes with a reported LOD value of 10 nmol/L. Subsequently, the procedure was applied to evaluate the formation of thiomolybdates from molybdate and sulfide with time, the stability of tetrathiomolybdate during storage as well as the occurrence of thiomolybdates in natural waters. It was found that the presence of a high excess of sulfide to Mo at neutral pH favours the formation of thiomolybdate. Concerning sample preservation, the authors highlight that sample acidification could lead to species transformation and losses of Mo by precipitation so flash freezing is recommended as the most suitable storage method. Dithiomolybdate, trithiomolybdate, and tetrathiomolybdate were detected in approximately half of the 48 spring water samples collected from the Yellowstone Park, constituting 20%, 12%, and 38%, respectively, of the total Mo.

4.11 Platinum

Analytical studies into the *interaction of Pt-containing drugs with different cellular components* continue to be undertaken, providing new approaches to a much investigated area, although with little actual clinical relevance. A detailed study[36/11387] to address differential cellular uptake of pyrodach-2, a new candidate Pt-drug, was conducted in three different cell lines. The normalization of Pt results to cell mass, after freeze-drying, was used to minimize the errors associated with cell counting. Similarly, Pt accumulation in DNA was evaluated by referencing the Pt results to the DNA concentration, as measured by ^{31}P monitoring using flow-injection and SF-ICP-MS detection. The adducts formed with a model single-strand length of DNA were qualitatively evaluated after enzymatic hydrolysis using Nuclease S. A capillary C_{18} HPLC column connected either to a SF-ICP-MS or ESI-Q-TOF-MS was used to identify the DNA Pt-adducts and showed the main adduct formed was, as would be expected from the many years of research in this area, with a GG dinucleoside. Unlike similar work by others in this area, but not referenced in this paper, the concentration of each adduct in the cell lines were not determined and the system was only applied to a model DNA system, rather than real patient samples. One wonders when this type of navel gazing will become less prolific.

4.12 Phosphorus

Due to the current restrictions on the use of certain hazardous substances in electrical and electronic equipment, phosphorus-based flame retardants are being developed instead of those containing bromine. For this reason some *candidate reference materials (RM) for the analysis of phosphorus-based flame retardants in styrene-based polymers* have been prepared[36/06872]. Blends of polycarbonate and acrylonitrile-co-butadiene-co-styrene as well as of high-impact polystyrene and polyphenylene oxide were chosen as carrier polymers, to contain the flame retardants resorcinol-bis-(diphenyl phosphate), bisphenol A bis(diphenyl phosphate), triphenyl phosphate and triphenyl phosphine oxide. Homogeneity and thermal stability of the candidate RMs were investigated and showed that the candidate RMs were comparable to the available industrial materials. Measurements by ICP-OES, FTIR and NMR confirmed the expected concentrations of the flame retardants and proved that analyte loss and degradation, respectively, was below the uncertainty of measurement during the extrusion process.

A 2D IC separation system coupled to ICP-MS using a heart-cutting mode for the *separation of organophosphorous compounds* was developed[36/09781]. The analytes were generated during thermal degradation of three different commercially available Selectilyte$^{(TM)}$ lithium ion battery electrolytes. The composition of the investigated electrolytes was based on lithium hexafluorophosphate ($LiPF_6$) dissolved in ethylene carbonate/dimethyl carbonate (50:50 wt%, LP30), ethylene carbonate/diethyl carbonate (50:50 wt%, LP40) and ethylene carbonate/ethyl methyl carbonate (50:50 wt%, LP50). The organophosphates were pre-separated from PF_6^- on a low capacity A Supp 4 column, which was eluted with a gradient step containing acetonitrile. The fraction containing the analytes was retained on a pre-concentration column and after that transferred to the high capacity columns, where the separation was performed isocratically. Different stationary phases and eluents were applied on the 2nd dimension for the investigation of

retention times and a suitable separation was obtained with a high capacitive A Supp 10 column. The organophosphates generated in LP30 and LP40 electrolytes could be separated by application of an aqueous NaOH eluent providing fast analysis time within 35 min. For the separation of the organophosphates of LP50 electrolyte due to its complexity a NaOH eluent containing a mixture of methanol/water was necessary. Inositol phosphates (InsPn) represent the dominant group of organophosphorus compounds in many soils and sediments, but they are rarely considered because the preferred method of analysis in environmental samples is solution ^{31}P NMR. In a recent study[36/05391], extraction of metal-associated InsP as a selective method for isolating and identifying inorganically bound InsP in environmental samples, was combined with HPLC-ESI-MS/MS for the identification and quantification of InsP in sediment samples. The chromatographic separation was carried out using water and ammonium bicarbonate as mobile phase in gradient mode. Data acquisition under MS/MS was attained by multiple reaction monitoring. The technique provided a sensitive and selective detection of InsPn in sediment samples. Several forms of InsPn in the oxalateoxalic acid extracted sediment were identified, InsP(6) was the dominating form constituting 0.250 mg g^{-1} DW as P; InsP(5) and InsP(4) constituted 0.045 and 0.014 mg g^{-1} DW as P, respectively. The LOD for the HPLC-ESI-MS/MS method was 0.03 □M InsP(n) which, unsurprisingly, is superior to the currently used NMR based methods and the sample handling time was also significantly reduced.

4.13 Ruthenium

Following on from last year's use of conjoint liquid chromatography (CLC) for the investigation of the interaction of Pt-containing drugs with serum proteins, it has now been applied[36/00639] to the *interaction of Ru-containing chemotherapeutic agents with serum proteins*. Two monolithic disks, CIM Protein G and CIM DEAE were assembled together in a single housing forming a CLC column. Isocratic elution with Tris-HCl-$NaHCO_3$ buffer (pH 7.4) in the first min, followed by gradient elution with 1 mol L^{-1} NH_4Cl (pH 7.4) in the next 9 min. The immunoglobulins (IgG) were retained by the Protein G disk enabling subsequent separation of unbound Ru species from Ru species bound to human serum Tf and albumin (Alb) on the CIM DEAE disk. Finally, elution with acetic acid (AcOH) in the next 3 min allowed separation of Ru species associated with IgG. Protein elution was followed online with UV detection at 278 nm, while the separated Ru species were quantified by post-column ID-ICP-MS. The instrumental set-up enabled fast 2D separation by affinity and ion-exchange modes to be carried out in a single chromatographic run. Two Ru-based chemotherapeutics: a newly synthesised compound chlorido(□^{6}-p-cymene)(nalidixicato-k^2-O,O)Ru(II) and (H_2im)[*trans*-Ru(III)Cl_4(Him)$_2$], which is currently undergoing preclinical studies, were investigated. The CLC procedure gave an LOD 0.027 □g Ru mL^{-1} for the unbound Ru species and good method repeatability (RSD 3.5%). The method enables investigation of the kinetics of interaction of positively charged and neutral complexes of metallodrugs with serum proteins. However, negatively charged metallic complexes co-eluted with Tf and HSA and thus hindered their speciation analysis. The interaction of another Ru-containing anti-cancer drug, indazolium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)], with the cytosolic proteins from colon cancer cell lines has been investigated using a shotgun proteomic approach[36/07612]. Using SEC-ICP-MS revealed that over 85% of Ru was converted into a high molecular-mass fraction, which was further investigated using

a shotgun approach, with the entire proteome being digested and the resulting peptides being analyzed by capillary HPLC combined with ESI-MS/MS. This allowed for identification of the ruthenated proteins on the basis of characteristic MS/MS spectra of the respective peptides. It was found that both Ru(III)- and Ru(II)-ligated functionalities participate in adduct formation, the hydrolyzed forms of the drug being attached to the majority of the binding proteins.

4.14 Selenium

A *review devoted to Se in contaminated waters* has been published[36/06841]. The reviewers deal with a range of topics that includes; an overview of selenium chemistry, toxicity and environmental impact, a description of analytical techniques used for selenium speciation including separation techniques coupled with spectrometric techniques, electrochemical techniques and non-chromatographic methodologies and an evaluation of existing and new emerging technologies for removing selenium from waters. There are extensive tables summarising the analytical methodologies for determining selenium species and the procedures for selenium removal from waters. A technical note which reports the advantages and disadvantages of ICP-MS and ESI-MS for selenium speciation in biological samples has been presented this year[36/07997]. The article includes a summary table with information on LOD values and sensitivity provided by ICP-MS and ESI-QTOF-MS for the determination of Se^{VI}, SeMet and TMSe. Based on their own experimental data, the authors underline that the most effective use of ESI-MS is for species identification in conjunction with ICP-MS for species quantitation.

A number of *methods for non-chromatographic selenium speciation* have been reported this year. An SPE method for Se^{IV} and Se^{VI} in water samples, based on the use of palladium nanoparticles (PdNPs) as the sorbent and ETAAS measurement, has been presented[36/10809]. The method implies the use of $NaBH_4$ for both the reduction of Pd^{II} to Pd^0 to obtain PdNPs and Se^{IV} to Se^0. The generated Se^0 was adsorbed on the surface of PdNPs and thus separated from Se^{VI}. Following adsorption, PdNPs were collected by centrifugation, dissolved in HNO_3 and the extracted Se quantified by ETAAS. Total Se in waters was determined after reduction of Se^{VI} to Se^{IV} by microwave heating and the Se^{VI} concentration was calculated by difference between Se^{IV} and total Se. The Pd and $NaBH_4$ concentrations, pH, temperature and volume of sample were optimised by using a 12-run Plackett-Burman factorial design. At an enrichment factor of 100, the LOD was 0.025 □g/L. The method was successfully applied to the determination of Se^{IV} and Se^{VI} in spiked samples of ground water, with recoveries in the range of 97-102%. The dominant Se species was found to be Se^{VI} when the methodology was applied to ground waters collected in the Punjab, India. Silica-coated magnetic nanoparticles (SMNPs), surface modified with 5-sulfosalicylic acid (SSA), have been synthesised and used in a SPE procedure for selenium speciation by CE-ETAAS[36/07092]. The extraction was based on the interaction between $–SO_3H$ on the surface of SSA-SMNPs and selenium species. The resulting Se-loaded nanoparticles were separated from solution with an external magnetic field followed by desorbtion of the extracted Se species with 0.5 mL of 0.5 mol/L of Na_2CO_3 solution. Subsequently, the selenium species were separated and detected by CE-ETAAS. For this purpose, an interface was developed for delivering Se species separated by CE to ETAAS. The detection limits for Se^{VI}, Se^{IV}, SeMet and $SeCys_2$ were 0.17, 0.17, 0.54 and 0.49 □g/L, respectively. The reported detection limit was 10 times lower than that found when the SSA-SMNPs were not used. The enrichment factors

and RSDs were in the range of 12-21 and lower than 5%, respectively. The method was applied to selenium speciation in fermented bean curd wastewater and juice. Only inorganic Se species were detected in the fermented bean curd wastewater, whereas Se^{IV}, Se^{VI}, SeMet, and $SeCys_2$ were found in fermented bean curd juice. The amounts of Se^{IV} and Se^{VI} in the fermented bean curd wastewater were determined as 2.62 and 3.83 ng/mL respectively whilst the Se^{IV}, Se^{VI}, SeMet, and $SeCys_2$ content in fermented bean curd juice were 4.08, 6.39, 2.77 and 4.00 ng/mL, respectively. Finally, a method for Se^{IV} determination based on UV-photochemical vapour generation followed by *in situ* trapping and atomization in a iridium-coated graphite tube is presented[36/10113]. Volatile selenium compounds were formed as a result of UV irradiation of a mixture containing selenite ions, formic acid and, in some experiments, HNO_3. Under optimal conditions, LOD values of 4.1 and 5.7 ng/L were achieved for Se with and without adding HNO_3 to the reaction mixture, respectively. The proposed method was successfully applied for the determination of Se in a simulated natural water (NIST SRM 1643e). The paper contains detailed discussions of the effect of different experimental parameters (role of formic acid and nitric acid, hydrogen flow rate and time and temperature of trapping).

The metabolism of *Se in plants* remains of research interest. The speciation of selenium in the roots of *Thunbergia alata* exposed 10 mM of Se^{IV} has been evaluated by HPLC-ICP-MS/ESI-MS and X-ray techniques[36/09300]. The Se species separation was performed using a Zorbax Eclipse XDB C-18 column (4.6 mm x150 mm, 5 mm, gradient mobile phase of 100% to 80% of 0.1% v/v aqueous formic acid and 0.1% v/v formic acid in methanol). The HPLC eluate was split after the column, 80:20 to the ESI-MS and to the ICP-MS. Up to 6 Se-containing species were detected, binding more than 80% of the accumulated selenium. Of these, three were identified as selenocysteinyl-2-3-dihydroxypropionylglutathione, seleno-phytochelatin 2 and seleno-di-glutathione. The XAS spectra of the root samples showed a structure similar to that of SeCys and elemental selenium (Se^0). The Se^0 in root tissues was quantified by developing a procedure to convert the Se^0 to selenosulfate ($SeSO_3^{2-}$) using 1 M SO_3^{2-} as an extractant solution and further analysis by AE-HPLC-ICPMS. The concentration of Se^0 found was approximately 20% of total selenium. A mass balance approach, which compared the sum of the Se species detected with the total Se found in root by a concentrated HNO_3 MAE digestion procedure, gave quantitative recoveries greater than 98%. In another report, a RP HPLC (Zorbaz 300A SB-C18 100 mm × 2.1 mm i.d., 3.5 µm) ESI-MS-MS method was developed and applied to identify seleno-compounds in Se-enriched soy beans[36/11096]. The Se concentration in soy beans ranged from 123 to 1120 mg/kg as measured by GFAAS. The Se compounds present were extracted by applying enzymatic hydrolysis with proteinase K to the soluble fraction of soybean proteins. HPLC-ESI-MS parameters such as capillary fragmentation voltage, collision energy and mobile phase composition were optimized in detail and Se species were monitored by using multi reaction monitoring (MRM) mode. The identification of selenoamino acids was performed by comparing the MS spectra of the sample with those of standards at the corresponding retention times. Under optimized conditions, the reported LOD values were 0.25 and 0.5 □g/L for MeSeCys and SeMet, respectively by using the MRM ion pairs 184–167 (MeSeCys) and 198–181 (SeMet). The main Se species found in Se-enriched cabbage was MeSeCys and this accounted for the 66% of the total selenium in the soluble protein fraction whilst SeMet was detected at a much lower concentration. Palomo-Siguero *et al*[36/07675] evaluated the

biotransformation of chitosan modified SeNPs (CS-SeNPs) in radish plants (*Raphanus sativus*) by using an analytical methodology which combined HPLC-ICP-MS, AF^4-ICP-MS and TEM. The CS-SeNPs were synthesised using a solution-phase approach based on the reduction of selenite with ascorbic acid in the presence of chitosan as stabiliser agent. Analysis of radish plant protease XIV extracts by HPLC-ICP-MS showed that the CS-SeNPS underwent transformation in the plant system. In radishes exposed to CS-SeNPs over a 95% of total selenium accumulated was found as MeSeCyst and SeMet. Characterization of diameter size in radish root system was performed by using both AF^4-UV-ICP-MS and TEM. The size distributions of CS-SeNPs in the in radish root systems determined by TEM were in good agreement with the values obtained from AF^4-ICP-MS. These results are of importance since the number of applications of AF^4-ICP-MS to diameter size estimation of nanoparticles in living systems is still scarce. The authors speculate that the CS-SeNPS are first adsorbed on the root system and then transformed into organoselenium compounds *via* a similar metabolic pathway to selenite. The possibilities of using XAS and XANES to study the affect Se-hyperaccumulators (*Stanleya pinnata and Astragalus bisulcatus*) on selenium speciation in soils and neighbouring plants (*A. ludoviciana, S. ericoides and C. album)* have been explored by El Mehadawi *et al*[36/07348]. Data evidenced that Se-hyperaccumulators and neighbouring plants produced similar XANES spectra, with both types of plant accumulating predominantly reduced selenocompounds providing a XANES spectra similar to the C-Se-C compounds SeMet and MeSeCys. The study suggests that the litter of Se-hypperaccumulators and the presence of microorganisms are among the sources of the presence of organic selenium in soil. Finally, the effect of selenium in the yield and seed quality of lentils (*Lens culinaris* Medik) of different genotypes has been investigated following Se^{IV} and Se^{VI} supplementation using either soil or foliar application[36/08739]. Analysis of protease XIV extracts by HPLC-ICP-MS found that SeMet was the main Se specie in lentil seeds. Adding increasing amounts of Se to the lentils resulted in increasing biomass, grain yield and seed selenium concentration, especially when employing foliar application.

There have been a number of *reports focusing on selenium speciation in Se-enriched food and supplements* this year. In one such study, cabbages were grown in pots containing peat fortified with selenium sodium salts (Se^{IV}:Se^{VI} (1:9)) at three levels of supplementation: 6 mg Se/kg, 21 mg Se/kg and 169 mg Se/kg MS[36/07015]. Samples of cabbage, both raw and cooked, were then subjected to an *in vitro* gastro-intestinal extraction, using amylase, pepsine and pancreatine and the resulting fractions subjected to HPLC-ICP-MS analysis. Total Se concentrations, for each peat enrichment level, were found to be 11, 98 and 960 and 12, 65 and 638 mg Se/Kg for raw and cooked Se-enriched cabbage, respectively. The main Se species found in raw and cooked cabbage were Se^{IV}, SeMet and Se^{VI}. The authors suggest that the results obtained show that the heat treatment and/or *in-vitro* gastro-intestinal digestion did not cause Se-species transformations. However, the results evidenced that boiling the cabbage increases the concentration of SeMet (from 5% to 32% with respect to the non-boiled cabbage) whereas Se^{VI} levels decreased (from 80% to 20%). An *in-vitro* gastro-intestinal digestion method was also applied to determine the bioaccessible fraction of Se in Se-enriched bread[36/03038]. The Se content in Se-white and Se-wholemeal bread was found to be 1.28 ± 0.02 µg/g and 1.16 ± 0.02 µg/g, respectively. The total Se bioaccessibility was 100 ± 3% and 40 ± 1% for white and wholemeal Se-enriched bread, respectively. Speciation analysis was performed in the gastrointestinal extracts, using RP-post column ID-ICP-MS, which revealed that Se

in the bioaccessible fraction from white bread was mainly present as SeMet, which accounted for 73% of the sum of total Selenium extracted. However, Se-Met was not detectable in the wholemeal bread extracts which suggests a low bioaccesibility of SeMet from wholemeal bread. This is of interest from the nutritional point of view as whole wheat products are generally consumed tdue to their health benefits. Finally, SR-XRF combined with isoelectric focusing (IF) gel electrophoresis has been used for identifying and quantifying selenium with a spatial resolution of several □m in Se-yeast[36/07956]. Selenium-containing compounds were first extracted and subsequent separated by 2-DE. After being separated, Se in 157 protein spots was analyzed *in situ* by SR-XRF scanning with minor damage to the original samples at the sub-□g g^{-1} resolution level.

A range of *applications concerning selenium metabolism,* based on the use of elemental and mass spectrometry and enriched stable isotopes as tracers, have been reported. Selenocyanate has been reported as a metabolite in human hepatome HepG2 upon exposure to selenite in a recent study by Anan et al [36/10504]. Analysis by HPLC-ICP-MS indicated the presence of Se in an unidentified molecule in the chromatograms of cell lines treated with selenite. Structural assignment was achieved by ESI-Q-TOF-MS on the basis of the agreement between the mass spectrum and the observed accurate mass of the unknown selenium metabolite and a commercially available selenocyanate. An increase in the peak area of selenocyanate was observed upon spiking the selenite treated cell extracts with a standard of cyanate, suggesting selenocyanate synthesis occurs through reaction between exogenous cyanate and selenite. Investigations into the bioavalability of selenocyanate in selenium-deficient rats (with depleted SelP and GPx levels) showed that selenocyanate and selenite behave in the same manner as the amount of selenium in SelP was fully recovered to the control level when either selenocyanate or selenite were administered. The antagonistic interaction between mercury and selenium in mouse (*Mus musculus*) has been evaluated[36/04831]. The protective effect of selenium on Hg toxicity after subcutaneous administration of 0.2 mg/kg body weight and per day of Hg^{II} and oral administration of 0.15 and 0.50 mg/kg body weight and per day of Se^{IV} was investigated over a period of 10 days using a metallomics approach. The combination of SEC-ICP-MS was used to characterize the metal-biomolecules distribution in liver, kidney and serum extracts. The use of an affinity column combined with species specific IDA provided information about selenoproteins distribution and selenium quantification. The study showed that selenoproteins protect against Hg either by binding mercury through the selenol group and by scavenging the reactive oxygen species induced by Hg. Several papers dealing with the determination of selenosugars have appeared this year. Iglesias *et al*[36/01475] have reported the identification of the selenosugar methyl 2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside (SeGalNAc) in liver and kidney tissues of lactating rats fed with formula milk supplemented with ^{77}Se. The liver and kidney cytosolic fractions were first fractionated in a Shodex Asahi Pack GS-520 HPLC column coupled on-line to the ICP-MS, and the low molecular weight-containing Se molecules were subsequent analyzed by HPLC-APCI-MS/MS. Based on the MS data, SeGalNAc was identified in liver and kidney with the highest values for maternal feeding rats (kidney 23 ± 3 ng/g; liver 26 ± 3 ng/g) compared to supplemented feeding rats (kidney 9.9 ± 0.3 ng/g; liver 10± 4 ng/g) and to non-supplemented rats (kidney 3.4 ± 0.5 ng/g; liver 4 ± 1 ng/g). A method to determine two selenosugars (SeSug1 and SeSug3), TMSe and MeSeCys in serum and urine of volunteers treated with five selenium supplements (Se^{IV}, Se^{VI}; SeMet, MeSeCys and Se-yeast) has

been reported[36/01609]. Analytical determinations were performed under four different sets of LC conditions with Se detection by ICP-MS. Separation of TMSe and SeMeSe, and SeSug3 was achieved by using two cation exchange (PRP-X200, 4.1×250mm) and (Ionospher, 5C, 3×200mm) columns, respectively. Anion Exchange chromatography (Hamilton PRP-X100, 4.6×150mm) was applied for Se^{IV} and Se^{VI} separation whereas reversed-phase chromatography (Atlantis dC18, 4.1×150mm) was used for detecting SeSug1 and SeMet. Moreover, MS-MS spectra were recorded to confirm the identity of each Se compound by the characteristic fragmentation profiles. The LOQ value was 0.25 □gSe/L for all selenium species except TMSe (determined by HPLC-VG-ICP-MS), which had an LOQ of 0.025 □gSe/L. Analysis of the urine and serum samples showed that the selenosugars were the dominating species. In addition, the proposed method allowed authors to determine basal levels of TMSe in urine samples.

A good alternative to the existing methods for determining SeSug1, SeSug2 and TMSe in urine is the use of a high temperature liquid chromatography (HTLC) system coupled to ICP-MS[36/03171]. Separation of these species was achieved in less than 7 minutes by using a Hypercarb (100 mm × 4.6 mm ID, 5 mm) column at 80 ºC. The mobile phase consisted of ultrapure water with 2% (v/v) methanol, flowing at a 1.2 mL/min. The LOD values were between 0.3–0.5 ng Se mL^{-1} with an RSD of 3% (n=5). The method was applied to urine analysis in two volunteers before and after the ingestion of Brazil nuts and supplements. As expected, the content of SeSug1 in urine significantly increased after selenium ingestion but concentration variability between individuals was detected. SeSug1 was the major urinary metabolite after selenium supplementation, representing 55–65% and 35–45% of the total selenium after the consumption of Brazilian nuts and supplements, respectively. This is reported by the authors to be the first paper on the determination of $SeSug_1$ and $SeSug_2$ in urine by HTLC-ICP-MS with the advantages of simplicity, short analysis time and the use of water as mobile phase. Finally, in a comprehensive study SeSug1 and selenoneine were determined in water extracts of fish muscle[36/10805]. Water extracts of fish samples were first pre-concentrated through preparative RP (Atlantis dC18 OBT 19×100 mm) column coupled to ICP-MS by using a mobile phase consisting of 20 mMol/L ammonium formate in 3 % methanol at pH 3.0, and a flow rate of 5 mL/min. The Selenium containing fractions were manually collected and then concentrated to a volume of 0.5 mL in a centrifugal lyophilizer. Subsequently, the pre-concentrated extracts were subjected to HPLC-ICP-MS and HPLC-ESI-MS analysis by means of a RP (Atlantis dC18 4.6×150 mm) column and isocratic elution using the same mobile phase at a flow rate of 1 mL min^{-1}. A major urinary excretion product of selenium, SeSug1, was identified for first time in fish muscle by comparing mass spectra of the unknown and SeSug1. In addition, selenoneine and the methylated derivative of selenoneine were also identified in fish muscle by accurate mass determination of fragments and molecular peaks. Undoubtedly, the detection of urinary Se metabolites in fish muscle opens a new perspective on the role of these compounds in Se metabolisms.

The metabolism of MeSeCys in rats by using $Me^{77}SeCys$ as tracer was evaluated by Sanchez-Martinez *et al*[36/00877]. For this purpose Wistar rats were fed with a diet containing $Me^{77}SeCys$-enriched sauerkraut. Analysis of major organ extracts by HPLC-IDA-ICP-MS showed a prominent increase (more than a 100% of Se level enhancement) of selenium in kidney and heart, whereas in

liver the selenium concentration only increased up to 20 % and remained constant in brain and testicles. It was also found that ^{77}Se-enriched sauerkraut supplementation did not alter the concentration of other essential elements in comparison to the controls, except in the case of heart and kidney, in which selenium was positively correlated with Mg, Zn, Cu and Mo. In addition, HPLC-ICP-MS analysis of hydrolyzed extracts after carbamidomethylation of the ^{77}Se labeled organs detected the presence of ^{77}SeCys. Anan *et al*[36/00031] have investigated the metabolic behavior of inorganic selenium and selenoamino acids in quails following an oral dose of 10 mg of $^{77}Se^{IV}$ and ^{77}SeMet. The seleno sugar SeGalNAc was excreted in urine, as determined by HPLC-ICP-MS and HPLC-ESI-MS, after the administration of both Se^{IV} and SeMet. The highest level of ^{77}Se-labeled compounds in the organs was found when selenium was supplemented as ^{77}SeMet after three days of treatment. Different combinations of chromatographic techniques and mass spectrometry have been used for identifying and quantifying GPX in human vitreous humor samples[36/04714]. Samples were fractionated by SEC-ICP-MS. The two Se-containing SEC fractions were identified as a GPX monomer (M, 22 KDa) and a tretramer (M, 88KDa) as confirmed by MALDI-QTOF-MS. The GPX was subsequently quantified by post column isotopic dilution analysis SEC-ICP-MS. The antioxidant enzyme activity was assayed for the two separated GPx species. For GPx (T) a linear relationship between activity and total Se concentration was found. Weekley *et a*l[36/01081] analyzed different organs (liver, kidney, heart spleen and testis) of selenium supplemented rats (at a concentration level of 1 and 5 mg/L as Se^{IV}) using XAS to determine the metabolisms of selenium uptake. Bulk XAS showed that Se-Se and Se-C species were detected in kidney and liver while Se-S species were only detected in liver. The Se K edge XAS spectra combined with principal component analysis (PCA) evidenced that of all organs closely matched each other, expect for liver and kidney, and closely resembled other models of selenium metabolisms such as human lung cancer cells.

In an outstanding study[36/00984], the operating conditions of *multi-isotope imaging mass spectrometry (MIMS)* were optimized with human aortic endothelial cells (HAEC) exposed to isotopically enriched selenium tracers for future applications to quantitative imaging the location of selenoproteins in cells. Cells were exposed to 150 nmol/L $^{82}Se^{IV}$, 600 nmol/L ^{77}SeMet and 300 nmol/L Met^{76}SeCys and subsequently grown in either well plates or on an Au-coated silicon chip (5mm× 5 mm). After cell growing, cell preparation involved freezing in liquid nitrogen and a fixing treatment with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer was compared. The following masses ^{12}C, $^{12}C^{14}$N, $^{12}C^{15}$N ^{28}Si, ^{32}S, ^{76}Se, ^{77}Se, ^{80}Se and ^{82}Se were simultaneously detected by using a NanoSIMS50L. It was found that all three selenium isotopes were mainly distributed in the nucleus as well as in the cytoplasm. The cell preparation methods tested were shown to be complementary. Freeze drying of cells allowed the analysis of the entire cellular volume in three dimensions while fixation retained selenium incorporated into proteins. The results confirmed the applicability and the great potentiality of MIMS in exploring the biosynthesis of selenoproteins in cells.

A study to determine the levels of *organic and inorganic selenium species in serum of 50 adults* (aged from 35 to 70 years) during a 30 month period has been undertaken[36/08780]. The total Se and Se species content in serum were measured by ICP-MS and HPLC-ICP-MS, respectively. A 90% of the studied subjects showed Se levels ranging from 95 to 161 □g L^{-1} with a mean value of 122.5 □g L^{-1}.

Se concentrations resulted to be lower in the youngest individuals (<50 years) compared with the older ones, in females compared with males, and in subjects with a body mass index of over 25 compared with leaner subjects. The majority of selenium was found to be present as organic species.

A paper reports *on the mechanisms of Se removal contaminated water* using granular Fe^0 as a permeable reactive barrier[36/11932]. For this purpose, batch experiments consisting on the anoxic reduction of Se^{VI} by granular Fe^0 in ultrapure water and in $CaCO_3$-saturated simulated groundwater were conducted. Selenium speciation and isotope analyses were measured by AEC-ICP-MS and XANES, and HG-MC-ICP-MS, respectively. After 3 days of treatment, AEC-ICP-MS revealed that only Se^{IV} was detected in $CaCO_3$ saturated simulated ground water while XANES evidenced the presence of Se^{IV}, $Fe_2(SeO_3)_3$, FeSe, $FeSe_2$, and Se^0 on the Fe^0 solid phase. In contrast, the forms of Se more reduced than Se^0 were not found in the solid phase in absence of $CaCO_3$. Stable isotope fractionation measurments by HG-ICP-MC-MS gave values associated with the reduction of Se^{VI} of 4.3 ‰ and 3.0 ‰ relative to NIST SRM 3149 with and without the presence of $CaCO_3$, respectively, which are larger values than those reported in the literature for sorption on Fe mineral (0.8 ‰). The reduction of Se^{VI} to Se^{IV} was speculated as the main cause of isotope fractionation, however, the potential effects of isotope equilibrium between adsorbed species and solution were not determined since the extent of fractionation is reported to be highly dependent on the water chemistry and the extend of Fe^0 weathering.

Two papers have been published that describes techniques based on sample introduction by HG approaches. A method has been developed for the determination of Se^{IV}, SeMet and SeCys in seafood based on the separation of selenium species by anion exchange (Hamilton PRP-X100, 4.6×150 mm) chromatography, followed by hydride generation and then detection by AFS[36/10358]. Factors affecting the separation and HG conditions were investigated in detail. The precision (RSD, n=10) was less than 5%. Recoveries from 87.3 to 103% were obtained for Se^{IV}, SeMet and SeCys in seafood samples. The LOD values of 1.6, 0.90 and 1.10 mg/L were obtained for SeCys, SeMet and Se^{IV}, respectively, which were low enough to detect either species in seafood samples. Unfortunately, details about SeCys integrity preservation during sample treatment are not given which make it difficult to properly evaluate the validity of the reported data. Similarly, Xiao *et al*[36/02065] used an HPLC-HG-AFS (Hamilton PRP-X100, 4.6×150mm column and a 20 mmol/L $(NH_4)_2HPO_4$ mobile phase) method for determining Se^{IV}, SeMet, MeSeCys and Se^{VI} in selenised-yeast. Selenium was completely released from Se-yeast by a protease and trypsin based extraction procedure. The results showed that the method offered good recoveries (83-101%) for Se-enriched yeast, with LOD values for the four species ranging from 0.5 to 5.0 mg/Kg.

Table 4 shows selected examples of other applications of Se speciation presented in the literature during the time period covered by this ASU.

Table 4 Applications of Speciation Analysis: Se

Analyte species	Technique	Matrix	Sample treatment	Separation	LOD	Validation	Reference
iSe, SeMet	ICP-MS m/z 77, 78, 80 and 82, collision cell H_2 or He	soil	MAE of 150 mg ("finely ground") with 5 mL 0.1 M NaOH in polypropylene tubes shaken at 250 rpm (sic) for 24 h centrifuged at 1500 rpm for 30 min	HPLC. Anion-exchange Hamilton PRP-X100, with 0.010 M ammonium citrate at pH 5.2, isocratic,	Not given	none	36/07797
iSe, Se-Cys2, SeMet,	ICP-MS m/z 80 collision cell CH_4	Chinese medicines: burdock, panax notoginseng	USE (53 kHz at 37 ºC) 500 mg (vacuum dried, ground to 100 mesh) with (a) protease and lipase for 1.5 h or (b) water	HPLC. Anion-exchange Hamilton PRP-X100, 0.080 M citric acid pH 5 (with NH4OH)	0.03 – 0.2 µg L^{-1}. Compound dependent responses shown by slopes of calibrations	Spike recovery	36/10572
iSe, Se-Cys2, SeMet, SeMet selenoxide,	ICP-MS m/z 78, 80 and 82, 78 preferred for samples with high	Fish and marine organisms	A. Enzymatic extraction of 200 mg with 2.5 mL	HPLC. RP ion-pair. XBridge C18, gradient, A: 0.0025.M (NH4)2HPO4 + 0.005 M TBAH	0.3 – 0.7 µg L^{-1}	NIST 1566b (oyster tissue), spike recover	36/01309

SeMeSeCys	bromide concentrations collision cell H_2		protease XIV (8 g L^{-1}) incubated in a tube rotator (24 h at 21 ◦C). B. 0.001 M $(NH_4)_2HPO_4$	+ 3% methanol pH 6.5 B: 0.005 M $(NH_4)_2SO_4$ + 3% methanol, pH 6.5		y	
iSe, Se-Cys2, SeMet,	ICP-MS m/z 77 78, collision cell H_2	Rainbow trout	Fish food: 100 mg extracted with 5 mL of a 0.1 M NaOH shaken for 20 h at room temperature. Centrifuged (2600 g, 10 min). Fish: ground with Milli-Q water in an Ultraturrax t25. SeMet: 100 mg extracted with 5 mL 0.1 M TRIS-HCl, pH 7.5, + 10 mg of protease	HPLC anion-exchange (PRP-X100), 5 mM ammonium citrate solution (pH 5.3) + 3% methanol, .and reversed phase ion pair (C8 Altima) C8, 0.1% heptafluorobutyric acid + 5% methanol	Not given	none	36/09595

			XIV for 20 h at 37 °C, centrifuged (2600 g, 10 min), incubated twice with 10 mg of protease, AE HPLC. SeCys: USE 100 mg with 2 mL 7 M urea in 0.1 M TRIS-HCl, pH 7.5), 7-step derivatization and clean-up, RP ion pair HPLC				
iSe, organic Se	HG-AAS. Method described previously	Dietary supplements	A. 0.100 M HCl B. 0.010 M Na_2HPO_4 + 0.045 M KH_2PO_4 (pH 6)	HPLC. AE Supelco LC-SAX1 (25 °C) phosphate buffer isocratic, post-column reduction of Se^{VI} thiourea	Not given	None	36/11806
SeCys, SeMet, Se-peptides and Se-proteins	ICP-MS m/z 77, 82	Se-enriched *Cordyceps militaris* (fungus)	Vacuum dried at -50 °C, ground, MAE 500 mg with 2 mLHNO_3	HPLC. RP ion-pair C18 5% acetonitrile Zorbax SEC 0.030 M Tris, pH 7.2	Not given	None	36/01001
Se^{VI},	ICP-MS m/z	Se-	USE 100	HPLC. AE PRP-	Not	NIST	36/082

SeMet	76,77, 78, 80. H_2 reaction gas	enriched wheat flour	mg with 3 mL of Tris-HCl + 0.020 mg Protease XIV, centrifuged (9000 rpm) for 20 min at 4 °C, filtered (0.22 µm Nylon)	X100, 0.010 M citric acid in 2% methanol, pH 5.0 (NH4OH). RP Zorbax Rx-C8 0.1% trifluoroacetic acid in 2% methanol	given	1567A (wheat flour), NRCC SELM-1 (Se-enriched yeast)	25
iSe, SeMet, SeCys2	ICP-MS m/z 78, 82, compound dependent responses	Dietary supplements	1. Sonicated with water, centrifuged (15,000 rpm) filtered (0.2 µm). 2 sonicated with 5 M HCl, centrifuged (15,000 rpm 20 min). 3. Shaken with 50 mg Protease 14 in 25 mL of 0.030 M Tris pH 7.2 for 24 h, sonicated, centrifuged, filtered.	HPLC. AEC Speris S5 SAX, gradient 0.005 M citrate in 10% methanol at pH 4.5 and 6.0. CEC Speris S5 CAX, isocratic 0.010 M pyridinium formate in 5% methanol at pH 2.12	0.7 – 1 µg L^{-1}	None	36/04497

4.15 Silver

The rapid increase in the use of Ag nanoparticles (AgNPs) in consumer products due to their antimicrobial activity has necessitated the development of methods for the speciation of the metal in different matrices. In this line, a method based on the use of hollow fiber flow field flow fractionation (HF5) to separate NPs of diameter size higher than 2 nm followed by a minicolumn packed with Amberlite IR120 resin (to trap ionic silver and NPs < 2nm) and on line coupled to several detectors (UV-VIS, DLS and ICP-MS) has been developed[36/10227]. The size characterization data obtained were in good agreement with that provide by TEM measurements. The applicability of the proposed method was evaluated by assaying it in spiked lake and river waters. The recovery of spikes of the seven Ag species added (Ag(I), the adduct of Ag(I) and cysteine, and five AgNPs with nominal diameters of 1.4 nm, 10 nm, 20 nm, 40 nm and 60 nm) ranged from 71 to 108%. The applicability of the method can be useful to the characterization of other type of nanoparticles. One article appears this year focused on the metabolisms and transformation of AgNPs in living system. Stegemeier *et al*[36/10197] evaluated the uptake and transformation of inonic silver (Ag^{I}), AgNPs and silver sulfide nanoparticles AgS_2NPs in alfalfa (*Medicago sativa*) hydroponically grown in presence of these three silver chemical forms. Total silver concentration analysis in root and shoots revealed that the majority (99%) of the plant-associated silver is sequestered on the roots irrespective of the chemical form of silver administered. However, XRF mapping evidenced differences in the distribution of Ag into roots. AgNPs are mainly accumulated in the (columella) border cells and elongation zone, whereas Ag^{I} accumulated more uniformly throughout the root and, Ag_2S-NPs remained largely adhered to the root exterior. Interestingly, TEM observation of roots shown the presence of AgNPs of smaller diameter size than the original added. Authors hypothesized the mechanism of Ag uptake from Ag-NPs as a combination of both NP attachment to the root surface followed by direct uptake of small or partially dissolved NPs into the root apoplast, as well as dissolution and Ag ion uptake.

4.16 Thallium

Three papers (two reviews and a research article) have appeared on *Thallium speciation*. The first review (77 references, 18 pages) covers Tl speciation in river waters[36/10174]. A considerable portion of the review is devoted to the sources and toxicity of Tl species and the methodology employed for Tl speciation (making special emphasis in the use of IC-ICP-MS and LC-MS as techniques which allow direct analysis of Tl species without requiring transformation steps). Useful summary tables including sample preparation methods are provided. Additionally, the review includes experimental data from the authors on the detection of $(CH_3)_2Tl^+$ and Tl^{I} in river water by employing IC-ICP-MS and/or LC-MS. For IC-ICP-MS analyses a C4 (4.0 x 250 mm) cation exchange column with 3 mmol/L HNO_3 as the mobile phase was used and an LOD of 12 ng L^{-1} for Tl compounds was obtained. The molecular structures of the detected Tl compounds were verified using LC-MS equipped with a Phenomenex Luna reversed phase column (150 mm × 3.0 mm, 3 µm) and by applying a gradient elution with mixtures of water:methanol. The study revealed the natural

occurrence of $(CH_3)_2Tl^+$ in river water. Four elements (As, Sb, Cr and Tl) are mentioned by JabBoNska-Czapla[36/04973] in a 103-reference (with titles) review on the use of LC-ICP-MS for analysing waters and sediment sample extracts. The review covers both sample preparation procedures and LC-ICP-MS methodologies for the mentioned elements. The contents of 103 articles are also featured in a summary table.

Finally, a solid phase extraction and speciation method for Tl^{I} and Tl^{III} species based on the use of *Chlorella vulgaris* as a sorbent and ETAAS has been proposed[36/10655]. It was found that Tl^{III} was selectively adsorbed in the biomass at a pH of 7.0 while Tl^{I} remained in solution. The total Tl was determined after oxidation of Tl^{I} to Tl^{III} with bromine water (1%). The Tl^{I} concentration was than calculated by difference. The LOD of the method was 8.3 ng/L of Tl^{III} with an enrichment factor of 50. The RSD for ten replicate determinations of 1 □g/L Tl^{III} was 5.1%. The method was successfully applied to tap, river, lake and reference water (natural water NIST SRM 1643e) samples. When the samples were spiked with 2□g $L^{-1}$of Tl^{I} and Tl^{III}, the recoveries varied between 96 and 103%.

4.17 Tin

Following the trend in recent years, there have been few totally novel studies involving tin speciation. Although the *determination of OTCs in sea water and seafood products* continues to be reported, most studies use well established techniques such as HPLC-ICP-MS. Other workers employ techniques which are strictly outside the scope of this review, although some may be of interest to those working in this area. One example is the work of Cui *et al.*[36/00179] who employed GC-MS-MS to determine six OTCs (MBT, DBT, TBT, MPhT, DPhT and TPhT), in seafood. The target compounds were extracted by hexane containing 0.01% tropolone, derivatizated by Grignard reagent n-PrMgBr, purified on a serial connection of silica and florisil SPE columns, and finally analyzed by GC-MS-MS. The LODs for the six OTCs were all below 0.1 µg Sn kg^{-1} in the case of wet samples, and were no higher than 0.5 µg Sn kg^{-1} for dry samples. The accuracy of the method was successfully validated using a mussel CRM (ERM-CE477, Mytilus edulis) and a spiked recovery test in four different biotic matrices, including tonguefishes, sea snails (Neverita didyma), scallops (Patinopecten yessoensis) and the Asian moon scallop, gave values within the range 70.5-105.3% for MBT, DBT, TBT, DPhT and TPhT, and 82.2-133.5% for MPhT. The RSDs ranged from 0.5% to 12.5%.

The speciation and quantification of the same OTCs (MBT, DBT, TBT, MPhT, DPhT and TphT) in sediment and drinking water by ID-HPLC-ICP-MS has been reported[36/08053]. All six species were eluted in less than 6.5 minutes with a binary gradient. Offline SPE was used to pre-concentrate the organotin compounds for quantification employing two calibration procedures; external standard calibration and isotopic dilution. The external standard calibration approach yielded LOD in the range of 1.5 to 25.6 ng L^{-1}. The method was linear over four orders of magnitude with regression coefficients greater than 0.99 and a peak area repeatability less than 4.5% RSD (n = 7) for all compounds. The ID method was three times more sensitive with LOD in the range of 0.5-1.2 ng L^{-1}. In contract to the more widely used GC-ICP-MS approach, no derivatisation was required. The methods were applied to sediments sampled from the Cooks River in Sydney.

The development, validation, and application of an *ultra-performance LC-SF-ICP-MS* method for simultaneous determination of MBT, DBT, TBT, MPhT, DPhT and TPhT in human serum has been

reported by Levine *et al*.[36/08398]. The BEH C18 column (2.1 x50mm^2 with 1.7 µm particle size) facilitated separation in approximately 3 minutes. The method was linear between 0.250-13.661 ng mL^{-1}, depending on the species. Interday precision (over 3 days) was less than 18%, and interday accuracy -14% to 15% for all species. Careful screening of reagents (most notably, tropolone) prior to use was essential in maintaining low back-ground levels of Sn. The method was then applied for the determination of the OTCs in human serum samples from women participating in the Snart-Foraeldre/MiljØ (Soon-Parents/Environment) Study in Denmark. The concentration of each OTC ranged from below the LOQ to 10.929 ng tin (Sn) mL^{-1} serum. Speciation values were confirmed by a total Sn analysis.

Two publications have reported on the *determination of TBT under the European Water Framework Directive* 2000/60/EC. The first of these overviews the development of a primary reference method for TBT[36/08629], whilst the second describes the experimental design for TBT quantification by ID-SPE-GC-ICP-MS[36/03411]. In the latter of these, the analytical process was optimised using a factorial fractionary plan. The design of experiment allowed the evaluation of 3 qualitative factors (type of stationary phase and eluent, phase mass and eluent volume, pH and analyte ethylation procedure) for a total of 13 levels studied, and a sample volume in the range of 250-1000 mL. Four different models fitting the results were then defined and evaluated with statistic tools prior to one being selected and optimised to find the best procedural conditions. C18 phase was found to be the best stationary phase for the SPE experiments. The study demonstated that SPE could be a robust and convenient technique for TBT pre-concentration at pico-trace levels.

Table 5 shows selected examples of other applications of Sn speciation presented in the literature during the time period covered by this ASU whilst Table 6 covers multielemental speciation methods.

Table 5 Applications of Speciation Analysis: Sn

Analyte species	Technique	Matrix	Sample treatment	Separation	LOD	Validation	Reference
Triethyltin, tributyltin,triphenyltin, azocyclotin	ICP-MS, oxygen flow 0.25 L min^{-1}, cooled spray chamber (-3 ^{o}C), m/z 120	seafood	MAE (500 mg) with n-hexane + ethyl acetate (1 + 1), centrifuge, evaporated to dyness, dissolved in methanol	HPLC. Pursuit 5 µ-C_{18}, RP gradient A: 10% glacial acetic acid pH 3.0 with triethylamine; B: methanol. 30 ^{o}C, 30	0.2 – 0.5 µg L^{-1}	Spike recovery, HPLC-tandem MS	36/02755

			(1 mL) Dispersive SPE with magnetic nanoparticles coated with octanoic acid (50 mg), vortexed (2 min), separated, filtered (0.22 µm)	min			
Seven OTC	ICP-MS	Aquatic products	USE with 30% acetic acid in methanol	HPLC. RP ion-pair, Eclipse Plus C-8, gradient methanol + acetic acid + 0.2% triethylamine	0.11 – 0.6 µg kg^{-1}	Spike recovery	36/09087
Six OTC	MS	Saudi coastal waters	SPE, propylation	GC, Agilent 19091Z 213 (30 m x 320 µm x 1 µm HP-1 methyl siloxane). Temp ramp of 12 ^{o}C min^{-1} from 40 to 300 ^{o}C	0.005 – 0.01 µg L^{-1}	Spike recovery	36/11247
OTC	MS	River sediment	Freeze dried (2 g), USE (30 min) with	GC, no details provided	0.2 – 0.6 µg	Spike recovery	36/07376

			0.03% tropolone in methanol (15 mL) + conc. HCl (1 mL.) Multi-step extraction, ethylation and concentration		kg^{-1}		

Table 6 Applications of Speciation Analysis for simultaneous speciation of multiple element species.

Analyte species	Technique	Matrix	Sample treatment	Separation	LOD	Validation	Reference
AsB, AsC, MMA^{V}, DMA^{V}, As^{III}, As^{V} iSe, SeMet, Se-Cys2	ICP-MS, m/z 75, 78, 82, collision cell He	Food (pork, chicken, rice and wheat)	Sliced, frozen, lyophilized, ground, powdered. USE of 1 g with 20 mL artificial gastric juice HCl + pepsin), centrifuged (8000 rpm 10	HPLC. RP Waters Symmetry C-18, 35 oC, with gradient 0.2% to 0.4% 1-butyl-3-methylimidazolium tetrafluoroborate in 5% methanol (pH 6.0)	0.3 – 1.5 µg L^{-1}	GBW10018 (chicken) and GBW 10043 (Liaoning rice),	36/11466

			min) filtered (0.22 µm)				
iAs, iSe, iTe	ICP-MS, ETV introduction, m/z 75, 77, 125	Environmental waters	Filtered (0.45 µm) and refrigerated (4 ^{o}C)	DLLME. As^{III}, Se^{IV}, Te^{IV} as DDTC complexes in bromobenzene	0.7 – 9 ng L^{-1}	GSBZ50004-88, GBW(E)080395, GBW(E)080548 (environmental water),	36/08437
MMA^{V}, DMA^{V}, As^{III}, AsV, Cr^{III}, Cr^{VI}, Cd^{II}	ICP-MS, m/z 52, 53, 75, 111, collision cell He	waters	Filtered (0.45 µm) and refrigerated (4 ^{o}C), EDTA complexes of Cr^{III} and Cd^{II},	HPLC, anion exchange, Hamilton PRP-X100, 0.040 M NH_4NO_3, pH 8.6	0.07 – 0.1 µg L^{-1}	Spike recovery	36/05633
iAs, iSb, iTl	ICP-MS, m/z 52, 53, 75, 111, collision cell He	River water	Not given	HPLC, Dionex IonPac AS-7 column. 30 ^{o}C Gradient A: 0.0015 M phthalic acid + 0.010 M Na_2EDTA (pH 4.5); B: 0.015 M nitric acid + 0.002 M diethylenetriaminepentaacetic acid	0.009 – 0.1 µg L^{-1}	Spike recovery	36/04210

5. Biomolecular Speciation Analysis

5.1 Direct Macromolecular Analysis

With the advent of SF-ICP-MS instruments and more recently triple quadrupole machines (QQQ-ICP-MS), the measurement of S and P is now achievable with reasonable LODs, which opens up the possibility for the direct measurement of biomolecules in the absence of metal(loid)s being present

in the molecule. A timely review[36/07608] (covering 167 papers) on this subject describes the *application of ICP-MS for S analysis with emphasis placed on sulfur-specific detection for hyphenated techniques such as HPLC, GC, CE and LA* coupled on-line to ICP-MS. Unsurprisingly, given the senior authors previous work in the area of ID-MS, the different approaches available for S isotope ratio measurements by ICP-MS are also detailed. Particular attention was paid to the quantification of peptides/proteins and the analysis of metallopeptides/metalloproteins via S by HPLC-ICP-MS. Likewise, the speciation analysis of metal-based pharmaceuticals and metallodrugs and non-metal selective detection of pharmaceuticals via S are highlighted along with labelling procedures for metabolic applications. The paper covers the instrumentation used for S analysis, including: Q-ICP-MS; CRC instruments; high resolution single and multi-collector instruments; as well as QQQ-ICP-MS. Finally, the measurement of natural variations in S isotope composition using multicollector ICP-MS was reviewed.

The *measurement of short lengths of single strand DNA and DNA-protein macromolecules, utilizing the detection of P and S* either by SF-ICP-MS or QQQ-ICP-MS illustrates the increasing interest in using the isotopes of P and S in biological and biomedical investigations. Antisense oligonucleotides are synthetically prepared and modified DNA fragments used therapeutically for the treatment of genetic disorders or infections. The most frequently studied group include the phosphorothioate oligonucleotides and their use requires accurate quantitation. A novel method[36/01952] investigated the use of ion pair ultra HPLC with a Hypersil GOLD C_{18} column containing a particle size of 1.9 □m (2.1 x 100 mm) and an eluent comprised of either 1,1,1,3,3,3-hexafluoro-2-propanol or triethylamine as ion pair reagent in water and methanol for their separation and SF-ICP-MS in medium resolution mode for detection. Monitoring ^{31}P was used to quantify the compounds at a concentration of 80 □g L^{-1}, while simultaneous determination of ^{32}S was useful for qualitative analysis. The results demonstrate the practical applicability of coupling ultra HPLC with ICP-MS in determining phosphorothioate oligonucleotides and their metabolites in serum within 7 min. The method was linear in the concentration range between 0.2 and 3 mg L^{-1} and the LOD was in the range of 0.07 to 0.13 mg L^{-1} depending on the length of the oligonucleotide. A new instrumental approach[36/04857] to investigate the interaction between proteins and DNA involved the removal of isobaric and polyatomic interferences from ^{31}P and ^{32}S using QQQ-ICP-MS. The system used state-of-the-art interference removal that captures ^{31}P in Q1, reacts it with O_2 in an octopole CRC generating ^{47}PO, allowing detection in Q3 without interference. Similarly, ^{32}S was reacted to ^{48}SO in the CRC, eliminating the polyatomic interferences at m/z 32. In combination with separation by HPLC this approach was used for the purification of a DNA-protein cross-linked product, offering potential for further studies investigating the interaction of proteins composed of the S containing amino acids Cys and Met, with DNA oligonucleotides.

5.2 Tagging and Labelling for Macromolecular Analysis

An insightful review[36/09428] covering *stable isotope labelling methods in MS based quantitative-proteomics* (looking at 164 papers) includes a section on the use of elemental tags for quantitative analysis by ICP-MS (covering 31 papers). Labelling methodology in general allows quantitative measurement of relative or absolute protein amounts, which is essential for gaining an insight into their function and dynamics in biological systems. Stable isotope quantitative proteomics allows

identification of equivalent peptides or peptide fragments by utilising the specific increase in mass due to mass tags with stable isotopes. The common workflow is to tag proteins or peptides with equivalent reagents, one of which includes a heavy mass tag and the other a light mass or no tag. The labelled samples are analysed by MS after being mixed and fractionated. The peaks in the mass spectra reveal the ratio of the two different isotopic or mass tag variants, which is then used to identify protein or peptide relative abundances. Several different strategies involving stable isotope labels were covered in the review, as well as label-free statistical assessment approaches and absolute quantification methods. The review describes the positives and negatives of the different stable isotope labelling methods which include metabolic labelling in live cells, ICP-MS based detection and post-harvest chemical label tagging for protein quantification. The section dealing with tagging methods used with ICP-MS detection included methods based on detection of the hetero-atoms such as I, P, S and Se, and chemical labelling using bifunctional chelating reagents, such as DOTA. The advantage of the second approach is that the label to be detected can be chosen, rather than having to rely on whatever hetero atoms are naturally present. This offers the opportunity to pick metal tags which have fewer interferences or for which the ICP-MS is particularly sensitive and it also allows multiplexing by labelling different molecules with different elements.

As mention above, macromolecules can be indirectly measured using atomic spectroscopy approaches by tagging or labelling the molecule with a metal or nanoparticle that makes the molecule "visible". This approach has produced some *novel applications for the measurement of serum proteins including Tf and homocysteine*, both important pathological biomarkers. An analytical method[36/11461] for the sensitive determination of homocysteine in human serum involved using ebselen, a Se-containing labelling agent to derivatize the reactive sulfhydryl group of the molecule in its "free" reduced form. Optimization of the derivatization and separation conditions by HPLC to isolate the excess of derivatizing reagent was carried out using UV/VIS detection and the Se labelling reaction was studied by using ESI-MS/MS, which showed a stoichiometry for the derivatization of 1:1. Quantification was accomplished by using post-column IDMS of Se in the serum samples, after precipitation of the main serum proteins and provided an LOD of 9.6 nM for real samples. A generic strategy[36/07253] for tagging proteins prior to quantitation by ICP-MS, was based on the use of Quantum Dots (QDs) as labels, which consist of a nanometer-scale core of a semiconductor material (CdSe), coated with an additional semiconductor shell (ZnS). In this strategy, streptavidin modified QDs (QDs-SA) were bioconjugated to a biotinylated secondary antibody (b-Ab_2). In order to develop a generic approach b-Ab_2 was designed to bind to the heavy chain of a primary antibody (Ab_1), such that the recognition capacity of Ab_1 against the target protein would not be significantly affected. Such a generic quantitative system, involving QDs-SA-b-Ab_2 could then be used against different protein targets just by changing the corresponding Ab_1 used. The targeted protein(s) were then quantified by ICP-MS determination of the Cd (or Se) present in the nanoparticle QD tag. After a multi-technique characterization of the synthesized QDs-SA-b-Ab_2 using GE and SEC-ICP-MS, it was used for the sequential quantification of five common serum proteins: Tf; complement C3; apolipoprotein A1; transthyretin; and apolipoprotein A4, at different concentration levels spiked into human serum samples. Results obtained were validated with those obtained using UV-vis spectrophotometry and commercial ELISA Kits. Using ICP-MS offered one order of magnitude lower LOD of 0.23 fmol absolute for Tf

compared to spectrophotometric detection, but the ICP-MS precision and detection limits were compromised by procedural blanks. The full analytical performance of the ICP-MS-based immunoassay proposed was assessed for detection of Tf, present at the low μg L^{-1} range in a complex "model" synthetic matrix, where the total protein concentration was 100 mg L^{-1}.

The *measurement of specific DNA targets* by adapting the conventionally used hybridisation chain reaction (HCR) amplification process by the addition of a metal label to one of the hairpin DNA probes via the use of a 1,4,7,10-tetraazacyclododecane-1,4,7-trisacetic acid-10-maleimidoethylacetamide (MMA-DOTA) linkage molecule has been investigated[36/08968]. The developed system used streptavidin (SA) conjugated magnetic beads to immobilize a biotinylated capture DNA probe (number 1) via specific biotin–streptavidin interactions. Target DNA, capture DNA probe number 1, and capture DNA probe number 2 form a sandwich construction because of their partially complementary sequences. Besides the complementary sequence of target DNA, capture DNA probe number 2 also contains an initiating sequence to trigger a chain reaction with two hairpin species one of which is labelled with a lanthanide, to form a nicked double helix. Hence, each target DNA molecule amplifies the signal by forming the double helix with hundreds to thousands of labelled hairpin molecules. After the magnetic separation process for removing excess DNA probes, the labelled hairpin molecules can be released from the magnetic beads into the solution via a simple thermal process. The ICP-MS signal from the solution correlated well with the target DNA concentration. By altering the sequences of capture DNA probe 1 and capture DNA probe 2, as well as lanthanide ion tags, different DNA targets can be detected simultaneously. In the current work a Tb ion was used as the label and a DNA sequence partially from human thymidine kinase 1 mRNA (TK1 mRNA) was chosen as the target. The system was calibrated in the DNA concentration range from 1 pM to 1 nM producing an almost linear correlation between the MS intensity and the logarithm of target DNA concentration. The LOD was estimated to be 300 fM which is comparable with previously reported rolling circle amplification (RCA) and ligase chain reaction based ICP-MS DNA assays and consistent with the several hundred to several thousand-fold signal enhancement normally provided by linear amplification approaches. Compared with the fluorescent dye labelled HCR DNA assay, the ICP-MS based assay did not show any improvement in sensitivity, probably due to the relatively low HCR efficiency in the magnetic bead surface. The main advantage of the assay is the possibility of quantifying many different metal labels and therefore DNA targets simultaneously, compared with the fluorescent labelling approach.

5.4 Metalloproteins, Metalloproteomics and Metallomics

As emphasised in this section previously[REF all previous speciation ASUs?], one of the main analytical stumbling blocks to be solved for an effective workflow in the analysis of metal containing biomolecules, is the *high resolution separation of intact metalloproteins without loss of the metal centre*. A timely and useful methodological review[36/02496] entitled “separative (sic) techniques for metalloproteomics require balance between separation and perturbation.” tries to address this subject. Covering 83 peer-reviewed papers it deals with metal losses induced by different separation systems including: gel electrophoresis; SEC and CE; RF-HPLC; IEC; and different separation materials. It also deals with metal losses inherent to a separation process for instance dilution and disequilibrium effects. In conclusion for metalloproteomic studies, the use of 2D

separations involving soft SEC followed by GE appears to offer the best compromise for achieving the required resolution without loss of the metal.

Novel work on separation methods for metalloproteins in this review period has been confined to *investigations in the area of electrophoresis*. If achieved, the coupling of GE to ICP-MS on-line clearly offers significant advantages because of the high resolving power of this separation mode for large biomolecules and the speed of analysis if it is coupled directly to the ICP-MS detector, rather than indirectly via LA analysis. A previously developed GE-ICP-MS separation system was applied[36/12054] to the quantitative investigation of the intracellular metal binding properties of two metallochaperones, HypA and HspA taken from *Helicobacter pylori* and over-expressed in *E. coli* cells. A modified column GE separation system (Bio-rad) was coupled with an ICP-MS spectrometer by casting a 3.0 cm long native gel in the column (8% and 15% gel were used for the separation of HpHspA and HpHypA protein lysates respectively), with a 0.5 cm long 4% native gel stacked on top. Tris–glycine running buffer (25 mM Tris,192 mM glycine, pH 8.3) was applied to the gel electrophoresis system, with 50 mM ammonium nitrate buffer transferring the eluted protein solution to the ICP-MS. The isotopes monitored included: ^{209}Bi, ^{59}Co, ^{57}Fe, ^{55}Mn, ^{95}Mo, ^{34}S and ^{66}Zn; Bi was included because the study went on to look at how this element affected the binding of metals to the two proteins. For protein identification, a T-piece was employed to split the solution after separation, transferring half of the solution to a collection tube. Protein fractions corresponding to the major metal peaks were collected and identified through peptide mass fingerprinting. The collected fractions were concentrated via ultrafiltration (Amicon Ultra, 3 kDa cut-off) and separated by Native PAGE. Protein spots of interest were excised from the silver-stained gels. After gel destaining and washing, the samples were digested overnight with trypsin at 37 ^{o}C. The digested peptides were desalted using Ziptips and dissolved in 0.1% formic acid and 50% acetonitrile. The molecular masses were determined by MALDI-TOF/TOF-MS analysis. The data were searched against the NCBI protein database using the MASCOT searching engine. The parallel detection of metal and S signals allowed for the accurate quantification of intracellular metal-protein stoichiometries, even for metalloproteins that bind metal ions with micromolar affinities.

5.5 Nanoparticle Speciation Analysis

An interesting development in this review period has been the instigation of atomic spectroscopy methods to investigate the number, size and composition of nanoparticles (NP), along with their interaction with biomolecules such as proteins. Nanoparticles are becoming more widely used in consumer products, pharmaceuticals and occurring as by-products of medical devices, and so the requirement for their measurement in environmental and biomedical samples is increasing. Over recent years the existence of "the protein corona," a natural interface between nanomaterials and living matter in biological systems, has evolved from a vague concept into an identified phenomena. The corona arises on the surface of all nanoparticles when they come into contact with physiological fluids and confers biological potency to the particles. This will clearly affect their cellular uptake, toxicological profile and ultimate fate within the cell or associated biological system and so methods for their characterisation would be beneficial.

Recent work[36/09551] using *ICP-MS analysis has characterised the protein corona* in a model NP

system resulting from the interaction of citrate-stabilized gold nanoparticles (GNPs) with human serum. The approach used was based on the assumption that the total protein content within the corona could be correlated to the S concentration resulting from the presence of cysteine and methionine amino acids. Once the most abundant proteins had been identified using a proteomic approach involving the use of GE and peptide analysis by ESI-MS/MS, the isolated NP-protein conjugates were measured by SF-ICP-MS measurement of the ^{32}S, ^{34}S and ^{197}Au content. Proteomics identified the 26 most abundant proteins that formed the corona with an average of approximately 40 S atoms per protein. Two conclusions could be reached for the system under the chosen experimental conditions: the number of proteins per GNP decreased with their size from 10 nm to 60 nm and the values obtained suggested that the protein corona in this specific case was theoretically formed either as a monolayer (60 nm GNPs) or as a multilayer (5 - 7 protein layers per 10 nm GNP). Clearly the work would not be applicable to real world samples as it included no method to isolate particles of a particular size, however it did show, once again, how the use of S : metal ratios could be used to characterise the interaction of macromolecules with inorganic systems. In a similar vein, but in this instance using CE-ICP-MS[36/10888] the interactions between serum proteins and GNPs were investigated, once again, in an *in vitro* study using Alb, Tf and HSA as model protein systems. Optimization of the system provided figures of merit such as migration time and peak area precision of 1.0 - 6.4% and 2.4 - 6.9%, respectively, LODs in the range of 0.8 - 1.0 □g L^{-1} and capillary recoveries on the order of 86 - 97%, depending on the NP size and conjugate type. The speciation of smaller particles (5 and 10 nm) was found to be dominated by Alb, with Tf, both in apo- and holo-form, playing the secondary role in developing the protein corona. For 20 and 50 nm nanoparticles, the contribution of Tf was initially comparable; however, with time it was replaced by Alb. In a real world study[36/07576] asymmetric flow field-flow fractionation (AF^4) coupled with ICP-MS was used to investigate metal protein binding and the size and composition of wear metal particles present in serum and hip aspirates from metal-on-metal hip replacement patients. A well-established AEC separation system using a Mono Q 5/50 GL, 50 × 5 mm,10 µm (GE Healthcare Sciences) coupled to ICP-MS was used to confirm the metal-protein associations in the serum samples and single particle ICP-MS analysis was used to confirm the approximate particle size distribution indicated by AF^4 in the hip aspirates. In the serum samples, AF^4-ICP-MS showed Cr was associated with Tf and Co with Alb and an unidentified species; AEC-ICP-MS confirmed these associations and also indicated an association of Cr with Alb. In the hip aspirate sample, AF^4-ICP-MS showed that Cr was associated with Alb and Tf and that Co was associated with Alb and two unidentified compounds; AEC analysis confirmed the Cr results and the association of Co with Alb and a second compound. Enzymatic digestion of the hip aspirate sample, followed by separation using AF^4 with detection by UV absorption (280 nm), multi-angle light scattering and ICP-MS, showed that the sizes of the Co, Cr and Mo containing wear particles were in the range 40 - 150 nm and single particle ICP-MS confirmed these findings.

5.6 Elemental and Molecular Imaging

The use of LA-ICP-MS for clinical imaging in different tissues and other biological materials, including liver, brain and kidney stones has been a focus of recent work. Imaging of Cu and other elements in experimental and clinical samples related to Wilson's disease using LA-ICP-MS has been

developed[36/04546]. Wilson's disease is an autosomal recessive disorder in which the liver does not properly release copper into bile, resulting in significant copper accumulation in various tissues. Affected patients suffer from hepatic disorders and severe neurological defects. Experimental studies in mutant mice models similar to Wilson's disease, in which the Cu-transporting ATPase gene (Atp7b) is disrupted, revealed a drastic time-dependent accumulation of hepatic Cu. The developed LA-ICP-MS method allowed for quantitative metal imaging in human and murine liver tissue with high sensitivity, spatial resolution, specificity and quantitation. For metal imaging 30 □m thick tissue cryosections were prepared and for quantitation, matrix-matched laboratory standards were prepared by dosing each analysed element to pieces of homogenized tissue. As a surrogate of slice thickness, the metal intensities were normalized to the average ^{13}C ion intensity. Interestingly the study showed that the age-dependent accumulation of hepatic Cu is associated with a simultaneous increase in Fe and Zn, while the intra-hepatic concentration and distribution of other metal(loid)s is unaffected. The same findings were obtained in well-defined human liver samples that were obtained from patients suffering from Wilson's disease. The signal for S was also measured, but no information was presented to describe how any potential interference was minimised. It would be interesting to repeat the work using a SF or QQQ ICP-MS instrument so that ^{32}S could be more accurately evaluated, to determine whether S is co-located with Cu and Zn, which might indicate the type of protein present and offer some speciation information. In a study[36/04204] comparing laser microdissection (LMD) and LA ICP-MS, mouse brain was stained with U and then imaged using both techniques. The main advantage of LMD over LA was the much improved spatial resolution, achieving a lateral resolution of 4 □m compared to 13 □m for LA. Although this was a preliminary study, the authors thought the lateral resolution could be further improved using a more powerful laser system and a SF-ICP-MS, which would offer better sensitivity, with the possibility to do single cell imaging.

The development of *new approaches to the calibration of LA-ICP-MS in the biomedical area* have been reported. A study [36/07374] on the preparation of matrix-matched calibration standards for quantitative elemental mapping of kidney stones by LA-ICP-MS was developed using a 213 nm Nd : YAG laser. The method was applied to the imaging of Ba, Mg, Na, P, Pb and Zn distributions in a section of kidney stone containing phosphate and oxalate phases. Homogeneity of the calibration pellets was examined by SEM and LA-ICP-MS, which showed that areas of individual biominerals were homogeneous and their size was less than the size of the laser spot. The internal reference element used was Ca, which was present in sufficient concentration in the kidney stones studied. Pellets were prepared without any binder using powdered uroliths (kidney stones) consisting of phosphate, oxalate and urate phases. Despite the fact that the individual calibration pellets differed significantly in their proportion of mineral phases, a linear calibration over three orders of magnitude was obtained for Ba, Mg, Na, P, Pb and Zn, with regression coefficients >0.9955 and narrow confidence intervals. Quantitative LA-ICP-MS can be difficult to achieve because of the requirement for representative solid standard materials, so work[36/08205] reporting new approaches to the absolute quantification of proteins are of considerable interest. The measurement of Tf and Alb in human serum using non-denaturing (native) GE combined with LA-ICP-MS and calibration using suIDMS is an important development, once again made possible by the measurement of isotopes of S, this time using SF-ICP-MS in medium resolution mode. In order to

achieve a homogeneous distribution of both protein and isotopically-enriched spike, protein standards of Tf, Alb and human serum CRM (ERM-DA470k/IFCC) were separated by native PAGE and the protein gel strips were immersed in spike solution containing ^{34}S for 2 min, and then dried for 3 h. The signals for the S intensities and ultimately the ^{32}S:^{34}S isotopic ratio were measured by LA-ICP-MS. The results were in agreement with the certified value with good precision and low uncertainty (1.5 - 3%). Moreover, the application of suIDMS using GE-LA-ICP-MS has the potential to offer reliable, direct and simultaneous quantification of proteins after conventional 1D and 2D gel electrophoretic separations.