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Atomic spectrometry update. Elemental speciation review

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This is the fifth Atomic Spectrometry Update (ASU) to focus specifically on developments in elemental speciation and covers a period of approximately 12 months from January 2012. The International Union for Pure and Applied Chemistry (IUPAC) have evaluated speciation and provided a definition as follows: "speciation analysis is the analytical activity of identifying and/or measuring the quantities of one or more individual chemical species in a sample; the chemical species are specific forms of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure; the speciation of an element is the distribution of an element amongst defined chemical species in a system". This review therefore deals with all aspects of the analytical 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. As with all ASU reviews[1–5] the coverage of the topic is confined to those methods that incorporate atomic spectrometry as the measurement technique. However, in the spirit of meeting the needs of the subject, material is incorporated that is not strictly "atomic spectrometry". For the most part, such procedures are those in which some form of molecular MS is used for speciation measurements, often in parallel with an elemental detector. As the content of this Update shows, the field is now maturing as evidenced by the extent to which the speciation of particular elements or technique combinations have been the subject of review articles. However, it is becoming increasingly difficult to ascertain the analytical details of the methodologies applied in speciation analysis, particularly where the paper is published in an 'application' based journal.

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1 Topical reviews

In this review period *several texts containing chapters of interest* have appeared. Many of the volumes in the ten-volume set of the *Encyclopaedia of Mass Spectrometry*[6] are now available, including volume 5 devoted to Elemental and Isotope Ratio MS. This volume contains eight chapters, the first of which covers the topic of the application of ICP-MS to elemental speciation analysis. Volume 8, entitled Hyphenated Methods, appears to be focused entirely on the determination of molecular species predominantly by GC-MS and LC-MS. The topic of isotopic determinations is the subject of two books. In *Isotopic Analysis: Fundamentals and Applications by ICP-MS*,[7] there is a chapter devoted to the determination of trace elements and elemental species using isotope dilution ICP-MS. A text covering *Isotope Dilution Mass Spectrometry*[8] has recently been published. The first part of this tome covers the theory and fundamentals of IDMS whilst the second part covers specific applications including the emerging field of quantitative proteomics. A new book, *Mercury in the Environment: Pattern and Process*, integrates the various sciences involved in the study of mercury cycling in the environment from the atmosphere, through terrestrial and aquatic food webs and human populations.[9] The book contains a chapter concerned with chemical analysis of waters, sediments and biota for Hg and its species. A second chapter which may be of interest covers the use of stable isotopes in mercury research. The second edition of *Methods of Analysis of Food Components and Additives*[10] contains a chapter devoted to trace element determination and speciation that is 35 pages long and cites 371 references. A book entitled *Arsenic & Rice*,[11] although written for an agronomist readership, does contain an account of the chemical measurements that underpin studies in this area. Topics covered include the measurement of total As and As species in rice and rice products. The techniques covered include HPLC coupled with ICP-MS and AFS.

There has been a considerable increase in the numbers of *review articles covering aspects of sample preparation*, and a decrease in the numbers of original research articles published, compared with last year's review. Liquid–liquid microextraction was the subject of two reviews. The speciation of some selenium compounds is mentioned in connection with applications of liquid phase microextraction applications in food analysis;[12] however, as no titles of the 146 cited articles are provided, it is difficult to assess the extent of the coverage. In discussing the application of liquid-phase microextraction to the analysis of environmental and biological materials,[13] Han and Row cite 7 articles concerning elemental speciation analysis out of the total of 166 citations.

A number of writers have scrutinised *solid-phase extraction*. In a review specifically devoted to SPE for elemental speciation in environmental samples, with special reference to aqueous solution, Das *et al.*[14] presented quite detailed summaries of about 50 articles covering mostly the speciation of As, Cr, Hg, and V. Latorre *et al.*[15] reviewed the use of carbon nanotubes as solid-phase extraction sorbents prior to atomic spectrometric determination of metal species. Applications to speciation studies, mostly of the elements mentioned above, are included in the 140 articles summarised. Herrera-Herrera *et al.*[16] undertook a broader assessment of the use of carbon nanotubes in a 241-reference review of applications in separation science over a three-year period from 2009 to 2011. The topics described include the use of carbon nanotubes as solid-phase extraction and microextraction sorbents, as parts of membranes and in chromatography and electrophoresis. A table of applications to the SPE of inorganic analytes that covers about 40 articles is presented. The redox speciation of chromium using sorption-based systems is the subject of two review articles.[17,18] The former review focused only on SPE procedures for Cr, whilst the latter considers SPE as part of a broader range of non-chromatographic separation techniques for Cr speciation, which includes co-precipitation, dialysis and LLE. Even though the sample type is "restricted" to natural waters, 87 articles are cited in this review.

The *applications of UAE* have been reviewed[19] with particular emphasis on its "green" characteristics: amount and nature of solvents used, safety of operation, energy involved, time required, and waste production. The review includes a short section on speciation analysis. The replacement of the lengthy procedures of the original Westöö method for the dissolution of MeHg, (involving use of concentrated HCl, benzene and multiple extractions) by MAE or UAE with no organic solvents is cited as an example of the progress towards greener analytical methods.

Some *separation techniques for elemental speciation* have been reviewed with an emphasis on electrically driven separations. The only chromatographic technique to be reviewed is HILIC[20] with particular reference to applications in environmental analysis. Although metal complexes are highlighted in the introduction to the review, the applications described are limited to two articles concerned with the determination of Gd species and one article describing the determination of OTC, by ES ionisation MS. The reviewers point out that the number of publications describing methods involving HILIC is increasing and that preliminary clean-up with SPE is often a feature of the methods. A useful compilation of other reviews of HILIC is included among the 45 articles featured. Haider *et al.*[21] point out that the on-line coupling of GE with ICP-MS, which dates from 2005, is a powerful tool for the separation, detection and quantification of bio-molecules that has been applied to the determination of phosphorus in DNA, phosphoproteins, and phosphopeptides. The determination of Au in nano-particles, Fe in metalloproteins, I in aerosols, and applications to the study of cisplatin–oligonucleotide interactions are also highlighted. It was speculated that the reason that relatively few papers have been published reflects the lack of familiarity with the benefits of what is considered a "promising" methodology. Comparisons were made with the alternative techniques of (a) SEC with ICP-MS detection, and (b) the coupling of flatbed GE with ICP-MS by LA. Compared with SEC, the advantages accrue from (a) the much lower concentrations of salts in the running/elution buffers, (b) the higher flow rates and (c) the lower costs of the separation media. In contrast to the LA interface, there is no loss of analyte as the separation is directly coupled to the ICP

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detector, and it is not necessary to stain the separated components to locate them on the gel. The status of PAGE and LA-ICP-MS within the general area of metallomics has been examined in some considerable detail.[22] The reviewers identify seven limitations of the methodology and list at least one strategy for overcoming each of them, together with an appropriate citation or two. The main limitations addressed are the integrity of the protein–metal bond, contamination, and the difficulty of quantification.

Two reviews of *separations by CE* have appeared. Even though the separation of metallothioniens by CE might seem to be a rather limited topic, 186 articles of relevance were identified,[23] several of which describe methods in which the separated components were detected by ICP-MS. In a survey of the CE of inorganic species as described in the 2009–2010 literature,[24] the reviewers cover the topics described in 198 publications. During this two-year period, many tens of relevant review articles were identified, including two featuring ICP-MS. Elemental speciation is featured prominently in the review, as are applications to the separation of conjugates between metal NPs and biomolecules. Microchip electrophoresis was identified as an area that is growing in popularity. Miniaturisation is also the theme of a 53-reference review of "lab-on-valve" systems of sample handling for metal determinations.[25] However, all the speciation applications described are based on solid-phase extraction rather than chromatographic or electrophoretic separations. The majority of methods feature detection by atomic spectrometry rather than by electrochemistry or molecular spectrometry.

Several reviews of the benefits of *ICP-MS for element-specific detection* covered a range of different areas of application. Donard and co-workers[26] reviewed 70 articles concerned with the determination of species-specific stable isotopes separated by chromatography, either HPLC or GC, and detected by MC-ICP-MS. The important experimental parameters and data reduction strategies that affect accuracy and precision are discussed. All the applications are critically evaluated in terms of analytical characteristics, advantages, disadvantages and potential for future applications to environmental, geochemical, and bioinorganic studies. Profrock and Prange[27] have reviewed the challenges, solutions, and trends in the applications of ICP-MS for quantitative analysis in environmental and life sciences. The focus of the review is on both recent developments and capabilities of ICP-MS coupled with different separation techniques, including HPLC, CE, GE and GC. A significant amount of the first part of the review, which covers methodology, is devoted to IDA. The second part of the review covers applications, in which about one-third of the total of 202 references are cited. The reviewers, unfortunately, state uncritically that the ICP-MS has compound-independent responses, which is not true, particularly when used as a detector following LC separations. The titles of the journal articles cited are given, which makes the reference list particularly informative. A review of developments and applications in hyphenated techniques as tools for speciation analysis of metal-based pharmaceuticals that features ICP-MS quite prominently has also been published.[28] Methods in which ES-MS is used are also described. The review covers the contents of 179 articles in which separation by one of the major separation techniques, HPLC, GC or CE is described. The reviewers also state uncritically that ICP-MS demonstrates species-independent sensitivity. A review covering ICP-MS in drug development: bioanalytical aspects and applications, cites 166 references. The analytical potential, the quantitative bioanalytical aspects, the various modes of operation and the challenges of the application of ICP-MS to the life sciences were described in detail.[29] The reviewers also consider the future potential of the technique in regulated bioanalysis and drug development.

A two part review, 201 references in total, of *HG analytical atomic spectrometry has been authored by* Long and co-workers.[30,31] The first part of the review covered the basic concepts, both BH-based and non-BH-based, of the HG technique. The second part was concerned with the elemental and speciation analysis of hydride-forming and non-hydride-forming elements in real samples but the coverage is dominated by lengthy tables that summarise the application by analyte and sample type.

Several reviews of *elemental speciation of particular sample materials* have appeared. Most of which are concerned with food and beverages. Welna *et al.*[32] reviewed the speciation (and fractionation) of elements in tea infusions as described in 60 articles. The reviewers write, "unfortunately, the speciation and fractionation analysis of elements in tea is uncommon" and indicate that the most of the work relates to Al, and that as far as other elements are concerned, "unfortunately outcomes on this matter are scarce and inconclusive". This seems a fair assessment of the status of elemental speciation in tea extracts. In a much broader review of trace element speciation in food,[33] the elements were divided into 'toxic' and 'essential' categories based on an assessment of 68 cited articles. The coverage of each element (apart from Se) was rather brief; for example, there are fewer than 10 references to As speciation and the authors implied, incorrectly, that there is no speciated arsenic-in-rice CRM. Ruzik[34] reviewed what is referred to as the speciation of "challenging elements" in food by atomic spectrometry. The elements classified in this way (Co, Cu, Fe, I, Mn, Mo and Zn) are a subset of those considered to be "essential nutritive". The others included in this larger group are Cr, F, Ni, Se, and Si. The review assesses the role of relevant compounds of each element in human nutrition and summarises the relevant literature on speciation (about 40 articles) in a table, from which it can be seen that the separation technique most often applied is SEC. A considerable portion of the review is devoted to sample preparation and a total of 135 articles are cited. It is also pointed out that many papers purportedly describing speciation in foodstuffs are actually describing fractionation methods. Recent developments in the speciation and location of As and Se in the challenging sample of solid rice grain have been reviewed.[35] More limited information was provided about the location of Ge, which was included because germanic acid is an arsenous/silicic acid analogue. It was proposed that the location of germanium may help elucidate the mechanisms of arsenite transport into grain. Most of the 63 articles included featured X-ray methods with some covering LA-ICP-MS. It was pointed

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out that the lower cost and greater accessibility of LA-ICP-MS compared with synchrotron techniques mean that the former is likely to be increasingly used in spatial studies of cereal grains. However, the fast, three-dimensional XRF microtomography that will be possible with advances in synchrotron detectors opens up the possibility of studies of fresh rice tissues at the cellular scale in the very near future. The writers also state, erroneously, that there is no speciated arsenic-in-rice CRM. The elemental speciation analysis of ambient aerosol particles by on-line MS has also been reviewed.[36] The elements of interest were Cr, I, N and S. The field is clearly in its infancy (only 31 references are cited) and extremely challenging given the very limited amount of sample available. It is likely that progress in this area will be slow, even allowing for the sensitivity of MS detection using laser desorption or thermal ionisation sampling capabilities. Reviews of methods for determination of polybrominated diphenyl ethers in environmental samples[37] and of the applications of X-ray spectrometry to the determination of the chemical environment of elements in cultural heritage items[38] may also be of interest. In the former review, the emphasis is on sample preparation and, in particular the application of extraction techniques such as SPME, single-drop micro-extraction, dispersive liquid–liquid micro-extraction, MAE, cloud point extraction, and hollow-fibre, liquid-phase, micro-extraction. In the latter review, which is written in French, the writers highlight the potential of XAS to provide insights into chemical reactions that occurred during the object's fabrication, and about the mechanisms of any subsequent alteration of an artwork.

2 Sample preparation

Other than as described in the review articles discussed above in Section 1, there has been relatively little research activity concerned with sample preparation for elemental speciation, beyond the development of SPE procedures.

2.1 Analyte stability

Olivares *et al.*[39] described a method for the *speciation of Sb compounds in vegetables*. It was shown that the extractant chosen, 10 mM EDTA at pH 2.2 with UAE for an hour, gave the highest extraction yield and was compatible with the HPLC mobile phase. It also prevented the oxidation of Sb^{III} to Sb^{V} and stabilised other Sb species, such as $TMSb^{V}$. The total Sb extraction efficiency from spinach was only 50% and from carrots and onion was 50 to 60% and 54 to 70%, respectively. Only Sb^{V} was detected in three roots (onion and spinach) that represented 60–70% of the total antimony in the extracts.

In a study of the *speciation of As, Cr, and Se in ash and soil leachates*[40] analyte stability was evaluated by spiking. A variety of environmental and geological samples, including waters, deionised water leachates, and simulated biological leachates from soils and wildfire ashes, were analysed. It was found that refrigeration at 10 °C was better at preserving species than freezing, particularly when all species were present. None-theless, the researchers recommend that sample solutions and extracts should be analysed as soon as possible to "eliminate species instability and interconversion effects". Speciation analyses were conducted on deionised water leachates and simulated lung fluid leachates of ash and soils impacted by wildfires.

2.2 Extraction methods

There would appear to be relatively few new studies of the extraction of elemental species from solid samples. In a comprehensive review[41] (219 references) of the uses of *ionic liquids in separation science and MS*, about 90% of the citations refer to the separation science part and 10% of the citations refer to MS. There are no citations to work in which analytes have been extracted from a solid sample by an ionic liquid.

Both *mechanical agitation and UAE* were evaluated in the development of the method for the determination of Sb species in vegetables, mentioned above.[39] The method was developed using CTA-VTL-2, a CRM made from Virginia tobacco leaves, from the Institute of Nuclear Chemistry and Technology in Poland, that has particle size less than 80 μm, in 0.01 M EDTA. It was concluded that UAE (for 1 h) was superior to mechanical agitation, extracting 74% of the total Sb (312 ± 25 μg kg^{-1}). As the real samples were prepared by freeze drying, it is not clear whether the particle sizes are comparable. Only Sb^{V} was found, which, according to the researchers, is good news, as Sb^{V} is "a low toxicity species that is almost harmless to humans". However, this may not in fact be the case.

In research describing the *UAE of iHg and MeHg from fish tissue*, it was found that the addition of 0.02 M thiourea to the 2 M hydrochloric acid followed by sonication for 5 min increased the extraction efficiency for iHg to 100%.[42] Without the thiourea, only 20% of the iHg present was extracted.

2.2.1 Enzymatic extractions. No new work has appeared on enzymatic extractions in the current review period. Bendicho *et al.* point out[19] that the acceleration of enzymatic hydrolysis by the application of focused ultrasound is to be regarded as controversial, as some researchers found loss of activity due to denaturation.

2.2.2 Liquid–liquid extraction. As was discussed above in Section 1, *several reviews of LLE have appeared*, including one devoted to ionic liquids.[41] Mostly the emphasis has been on micro-extraction procedures.[13,43,44] Liquid extraction procedures for Cr speciation in natural waters have also been reviewed.[18]

A *three-liquid-phase extraction* procedure[45] has been developed for the simultaneous enrichment and separation of Cr^{III} and Cr^{VI}. By suitable adjustment of pH and sodium sulfate concentration, the analytes were extracted from water samples into either the di(2-ethylhexyl)phosphoric acid top phase through a cation-exchange reaction (Cr^{III}), or into the PEG (2000 molecular weight) middle phase through ion-pair formation (Cr^{VI}). Over 90% of the Cr^{VI} was separated within 5 min.

For the determination of *triethyl-lead and tributyl-tin compounds in human urine*,[46] the analytes were derivatised directly in the samples (10 mL) by tetraethyl- or tetrapropylborate and the derivatives extracted into hexane (1 mL). Effective phase separation was achieved by centrifugation and 1 μL

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was injected for separation and detection by GC-AES. The LODs reported were 0.05 μg L^{-1} and 0.48 μg L^{-1}, for Pb and Sn, respectively.

Procedures involving *cloud point extraction* of Mn species in tea infusions[47] and of Sb species in natural waters[48] have been developed. In the former, flavonoid-bound Mn^{II} was extracted at pH 5.0 with Triton X-100 and the remaining aquated Mn^{II} and weakly-complexed Mn^{II} in solution were both chelated with 8-hydroxyquinoline and preconcentrated with Triton X-100 by cloud point extraction. A preconcentration factor of 10–20 gave rise to an LOD by FAAS of 2 μg L^{-1}. The procedure was validated by spike recoveries from water samples, comparison of the results with those of an SPE procedure, and by the analysis of a CRM (BCR 278R, mussel tissue). The method showed that about 30% of the total Mn in several black teas was extracted of which about 95% was in the unbound state. In the latter method,[48] Sb^{III} was selectively extracted with APDC as a chelating agent into a phase enriched with the surfactant Triton X-114. Although the article is in Czech, it is possible to discern that the method was validated by the analysis of a CRM, TMDA-61 (fortified water from the National Water Research Institute of Canada) and that recoveries between 90 and 107% were obtained for Sb^{III} added to some real samples that already contained measurable concentrations of this species. Total Sb was determined by ET-AAS with slurry sampling of the nano-sized titanium dioxide on which the species had been adsorbed. This aspect of the work is the subject of a separate paper.[49]

A method involving the *DMLLE of Fe species from water samples* has been devised.[50] $Iron^{III}$ was selectively extracted as the complex with 8-hydroxyquinoline into chloroform with methanol as the dispersive solvent. For a sample volume of 5 mL the extraction efficiency was critically dependent on the extractant solvent volume, ranging from 0% at 150 μL to 100% at 200 μL to 50% at 250 μL. The enhancement factor, defined as the ratio of the slope of the calibration curve after and before extraction (for 200 μL of extractant), was reported as 15; an interesting result, as, following evaporation of the chloroform, the residue was dissolved in 0.5 mL of dilute nitric acid giving an enhancement factor on the basis of volumes of 10. Using FAAS for the determination, the reported LOD was 4.5 ng mL^{-1}. Total iron was determined after oxidation with hot dilute nitric acid, and hence Fe^{II} was determined by difference. The procedure was successfully applied to the analysis of some real samples that did, in fact, contain measurable amounts of the two species.

2.2.3 Solid-phase extraction. As has been the pattern for the past several years, there is continued activity in the development of elemental speciation methods in which the species are separated by selective extraction onto the surface of a solid adsorbent.

As might be expected, *several procedures for the determination of* Cr^{III} *and* Cr^{VI} *have been described*. For the determination of the species in the presence of another analyte, As, Cr^{III} was selectively retained on a cation-exchange (Hyper Sep SCX) column filled with benzosulfonic acid.[51] After elution with HCl, the analytes were determined by ICP-OES. Total Cr was determined upon direct nebulisation of the sample and the researchers proposed two speciation procedures: either (a) determine the Cr^{VI} remaining after removal of Cr^{III} by the column and determine Cr^{III} by difference or (b) elute and determine the Cr^{III} and determine the Cr^{VI} by difference. It is not clear which, if either, was preferred. The LOD for both species was 1 μg L^{-1} and for As was 5 μg L^{-1}. The method was validated by the analysis of a CRMs BCR 544 (lyophilised solution, certified for both species) and NIST 1643d. The method was also applied to three real water samples only one of which, a waste water sample, contained measurable concentrations of both species. A magnetic SPE method was reported[52] in which Cr^{III} was retained on nanoparticles of Fe_3O_4@ZrO_2 at pH 8–9. After elution with 3.0 mL of 0.5 mol L^{-1} HNO_3 the particles were separated by magnetic decantation and the Cr determined by FAAS. The enrichment factor was 25, leading to an LOD of 0.7 μg L^{-1}. The method was applied to the analysis of 7 real samples (lake water, river water, sediment, serum and urine) and the Cr content in 5 of these was below the LOD. Spikes of both species at single digit mg L^{-1} concentrations were recovered at 94–107%. A magnetic SPE method in which Cr^{VI} was selectively retained has also been devised.[53] The extractant used was tetraethylenepentamine-functionalised Fe_3O_4, 20 mg of which was added to the sample (250 mL) and the mixture stirred for an hour, followed by separation and dissolution in 0.5 M sodium hydroxide solution (2.0 mL). The enhancement factor was 125, as would be expected for 100% extraction and elution, and the FAAS LOD was 0.2 μg L^{-1}. The method was applied to 4 river water samples, all of which contained single digit μg L^{-1} concentrations of Cr^{VI}, and all of which could be analysed directly by GF-AAS. Two research groups have devised methods in which transition metal phosphates are the selective extractants for Cr^{III}. In one,[54] the sorbent was iron phosphate, which retained Cr^{III} at pH 5.9 from 2 mL of sample followed by elution with 200 μL of 0.1% hydrogen peroxide and 0.05 M ammonia solution. The measured enrichment factor was 8.7 and the GF-AAS LOD was 0.02 μg L^{-1}. Total Cr was determined after reduction of Cr^{VI} to Cr^{III} with hydroxylamine hydrochloride. The method was applied to the analysis of a CRM (GBW08608, trace elements in water), which is known to contain only Cr^{III} (at 30 μg L^{-1}), and to four water samples, only one of which contained measurable concentrations of both species, in which the concentrations ranged from 0.4 to 0.9 μg L^{-1}. In the second method[55] the adsorbent was nanometre zirconium phosphate and the conditions, which are not explicitly stated in the article, were such that an enrichment factor of 300 was obtained leading to a GF-AAS LOD of 0.002 μg L^{-1}. The method was applied to the determination of Cr^{III} and Cr^{VI} in two natural water samples, both of which contained measurable concentrations of both species (ranging from 0.3 to 8 μg L^{-1}), and a CRM water sample (GBW(E)080257 containing 100 μg mL^{-1} Cr^{VI} that was diluted 10 000 times). Total chromium was determined after reducing Cr^{VI} to Cr^{III} with 10% (m/v) ascorbic acid solution and the concentration of Cr^{VI} was calculated by difference. Also of interest here is a report of a procedure in which Cr^{III} (together with Cd, Cu and Pb) was separated and preconcentrated on activated carbon modified with rhodamine 6G.[56] Following elution with 1 M hydrochloric acid,

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the analytes were determined by ICP-OES with LODs reported of about 0.4 μg L^{-1}.

For the *speciation of iAs and iSb in waters*[57] the trivalent species were complexed with APDC and selectively retained on a mini-column of carbon nanotubes followed by elution in a small volume of ammonia solution. The elements were determined by electrothermal atomisation AAS with an atomiser impregnated with a tungsten salt, which, after suitable thermal treatment acted as a permanent chemical modifier. For a 50 mL sample, an enrichment factor of 250 was obtained resulting in LODs of 0.02 and 0.05 μg L^{-1} for As and Sb, respectively. The total elemental concentration was measured after reduction with ammonium iodide solution and hence the pentavalent species were determined by difference. The researchers evaluated nine different types of carbon nanotubes from several sources and concluded that non-functionalised, multi-walled tubes from Nanocyl were the best. The method was validated by spike recovery from 6 real samples, only one of which contained all four analyte species at measurable concentrations.

For the *determination of* Fe^{II} *and* Fe^{III} *in Antarctic ice samples*,[58] the Fe^{III} was retained selectively at pH 2 on the chelating resin, Ni-NTA Superflow. After elution, with 1 M nitric acid, the Fe was determined by ICP-MS with an LOD of 0.01 μg kg^{-1}. Total iron was determined after oxidation of Fe^{II} with hydrogen peroxide. Every precaution was taken to minimise contamination and all the sample handling was carried out inside a flow injection manifold. The method was validated by the analysis of spiked melted ice samples and applied to six real samples ranging in age from 1000 to 31 000 years, all of which contained measurable concentrations of both analytes. It was confirmed that Fe^{II} was stable with respect to oxidation by dissolved oxygen under the conditions of the analysis. However it was reported that the signal for the unretained Fe-containing components was not amenable to quantification, as this signal was broad and also contained contributions from other Fe species.

Two research groups have devised *speciation methods for* V^{IV} *and* V^{V}. A new material, aminopropyl triethoxysilane-functionalised silica, was prepared and extensively evaluated with regard to the thermodynamics and kinetics of binding of the analytes and potential interferents.[59] The material was selective for V^V at pH 2, but retained both species at pH 3. Following elution with 0.5 M thiourea in 0.2 M hydrochloric acid V was determined by ICP-MS with an LOD of 0.04 μg L^{-1}. The method was validated for the determination of V^V by the analysis of four types of water that had been spiked at concentrations up to 1000 μg L^{-1} and, for the determination of both species, by the analysis of three types of water that had been spiked at concentrations up to 200 μg L^{-1}. An SRM (NIST 1643e trace elements in water, certified for total V of 37 μg L^{-1}) was accurately analysed, but V species in real samples were not determined. In the second method,[60] V species were detected in seawater following retention on a Chelex-100 column followed by selective elution of V^V with 5 mL of 0.1 M ammonia and V^{IV} with 8 mL of 0.1 M nitric acid. The determination of the separated species was carried out by ET-AAS. For a 50 mL sample (loaded at 0.83 mL min^{-1}), the LODs were 0.2 and 0.1 μg L^{-1} for V^{IV} and V^V, respectively. The method was validated, by spike recovery from seawater samples at concentrations of 5, 7 and 15 μg L^{-1}, and applied to the analysis of three samples, in which only V^V was found, at concentrations ranging from 0.1 to 0.25 μg L^{-1}.

A procedure for the *separation of Hg species* has been developed and applied to the determination of iHg and MeHg in wine.[61] Several materials, described as core–shell imprinted sorbents, based on silica gel chemically modified with 3-(trimethoxysilyl)propyl methacrylate as support, were synthesised and extensively evaluated. The best performance was obtained with a material prepared by the copolymerisation of methacrylic acid and trimethylolpropane trimethacrylate in the presence of the Hg complex with 1-pyrrolidinedithiocarboxylic acid. In the analytical method, 20 mL of sample was passed at 2 mL min^{-1} though a column of this material. The iHg was retained and any MeHg passed through. Total Hg was determined after digestion with nitric acid, and hence the MeHg was found by difference. The column was washed with 10 mL acetonitrile and the retained iHg was eluted, with 2 mL of 0.1 M thiourea in 0.1 M hydrochloric acid, and quantified by CV AAS with external calibration with matrix-matched standards. The LOD was 0.02 μg L^{-1}, well below the limit of 5 μg L^{-1} set by the International Organisation of Vine and Wine. The method was applied to an unspecified number of Bulgarian red and white wines in which the Hg^{II} concentrations ranged from below the LOD to 0.1 μg L^{-1}, but in which no MeHg was found. The researchers did not report an LOD value for MeHg.

3 Instrumental techniques and developments

3.1 Developments in species separation

A continuous on-line separation procedure for ICP-MS has been developed for the *speciation of inorganic Cr and V* in waters.[62] A cation self-regenerating suppression device described both as an electrodialyser and an anion membrane separator was used. It was shown that the device is capable of removing all anionic species from the sample stream, including V^V and Cr^{VI}. As a result some isobaric overlap interferences that arise from other anionic matrix components, such as chloride, nitrate and sulfate were significantly decreased. Total Cr and V were determined after reduction to the cationic lower oxidation states by sodium thiosulfate solution, and V^V and Cr^{VI} were determined by difference. The method was validated by the accurate analysis of two CRM (GSBZ 50029-94 environmental water) and (GBW 08608a riverine water), and by spike recovery (at concentrations of 0.2 and 1.0 μg L^{-1}) from three samples, all of which contained both V species and Cr^{III} and one of which contained all four species. The LODs were 0.02 μg L^{-1} for V^{IV} and 0.06 μg L^{-1} for Cr^{III}.

3.1.1 Liquid chromatography. To determine the *inorganic species of As, Cr and Se in ash and soil leachates*, an ion-pair HPLC procedure was developed that separated the two species of each element from each other within 3 min.[40] The stationary phase was a 3 μm C-8 material and the mobile phase was 2.0 mM tetrabutylammonium hydroxide with 0.5 mM dipotassium EDTA at pH 7.6 containing 5% methanol delivered at a flow rate of 1.5 mL min^{-1}. The separation was isocratic at 35 °C. Several possible

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gases were evaluated to effect the collisional dissociation of $^{40}Ar^{12}C^{+}$ that interfered with the determination of Cr at *m*/*z* 52. It was concluded that nitrogen was best, although it was indicated that better detection limits could be obtained for As by adding oxygen and monitoring[75] $As^{16}O^{+}$ at *m*/*z* 91. The detection capability was quantified as "practical quantitation limits" (PQLs) defined as the concentration of "the lowest standard analysed that read back to within ±20% of the prepared value". For a 50 μL injection volume, the PQL for the As and Se species was 0.05 μg L^{-1} and for the Cr species was 0.1 μg L^{-1}. The article contains considerable discussion of the role of spike recoveries in assessing analytical performance, from which it appears that there were problems with some samples.

An *anion-exchange chromatographic procedure for the speciation of P oxoacids* in natural and waste water samples has been developed.[63] The most common phosphorus oxoanions in aquatic environments: orthophosphate, phosphite, hypophosphite, pyrophosphate and tripolyphosphate, were separated on a 150 mm × 4.1 mm Hamilton PRP-X100 column (5 μm particles) by gradient elution at 1.5 mL min^{-1}. The mobile phases used were (A) 4 mM NH_4NO_3 at a pH 2.2 in 2% methanol (used to increase the P signal and improve the chromatography), and (B) 200 mM of NH_4NO_3 at pH 1.8 in 2% methanol. The total run time was 17 min, in which the compounds were separated in about 12 min. An ICP-OES instrument, at 213.618 nm, was used as the detector and, for a 20 μL injection, the LOD were in the range 1–5 mg L^{-1}. The calibration curves showed significant differences in slopes indicating that some compound-dependent responses were present. The method was applied to two samples in which all species were below the LOD, except that of orthophosphate in an effluent waste, which was detected but not quantified. Spikes of 20 mg L^{-1} were recovered satisfactorily, but spikes of 5 mg L^{-1} were not.

What the researchers described as *multisyringe chromatography for mercury speciation*[64] was a low-pressure chromatographic separation of three Hg species on a monolithic C-18 column (50 mm × 4.6 mm) through which the mobile phase was propelled by a syringe burette used as a pump. The device contained 4 such burettes, one of which was used to deliver tin chloride solution to the eluent from the post-column photoreactor to generate the Hg vapour that was detected by AFS. The mobile phase program was described as "multi-isocratic," meaning that following the chromatographic separation of Hg^{II} and MeHg with mobile phase 1 (0.005% 2-mercaptoethanol in 0.24 M ammonium acetate at pH 6 mixed 99 + 1 with acetonitrile), the holding coil was filled with mobile phase 2 (0.005% 2-mercaptoethanol in 0.24 M ammonium acetate at pH 6 mixed 90 + 10 with acetonitrile), which was then delivered to the column to elute the EtHg. For a 600 μL injection volume, the reported LODs were 0.03, 0.11 and 0.09 μg L^{-1} for MeHg, Hg^{II} and EtHg, respectively. The method was validated by the accurate determination of MeHg in a CRM, DORM-2 (dogfish muscle), which is certified for this species as well as for total Hg. It was applied to the analysis of two ground water samples and a landfill leachate, none of which contained any of the analytes at concentrations above the LOD, but for which satisfactory spike recoveries at single-digit μg L^{-1} concentrations were obtained. The method was also applied to the analysis of three paediatric vaccines, one of which contained 69 μg L^{-1} of EtHg and another contained 0.56 μg L^{-1} of Hg^{II}.

Different *signal treatments to improve the detection power of HPLC-ICP-MS* have been compared.[65] The test system used was the separation of arsenic species As^{III}, As^{V}, DMA and AB by AEC with ICP-MS detection. The injection volume was 20 μL and with gradient elution the analysis time was 8 min. The data collection rate was such that 1889 data points were collected for each chromatogram. The benefits of de-noising the signal by (a) convolution with Gaussian distribution curves, (b) Fourier transform, and (c) a wavelet transform method were examined. It was found that, although all methods improved the LODs, the best was the wavelet transform method, whereby the detection power was improved a factor of about 6. In the case of As^{V}, the species with the poorest LOD, the improvement was from 0.72 to 0.12 μg L^{-1}.

3.1.2 Gas chromatography. The *possibilities for compound-independent calibration with AED* has been evaluated[66] for several N and S compounds. The effect of column pressure and the roles of analyte concentration and molar mass were studied and it was found that inter-element response factor ratios, particularly C : S ratios, were very dependent on the molecular mass and concentration of the analytes, whereas molecular structure had less effect. The main conclusions were that calibration should be performed with a compound of similar concentration level and with a comparable molar mass to those of the analyte. When this could not be achieved the calibrant should have a retention time similar to that of the unknown. A strategy was proposed in which a mixture of reference compounds at appropriate concentrations covering the range of retention times was injected. From the resulting chromatogram, the most suitable compound for calibration of an unknown analyte could be identified. The researchers also showed the results of empirical formula calculations (C, H, and N or S) for 7 compounds, ranging from ethyl disulfide (C_4) to anabasine (C_{10}), each of which had been obtained by calibration with a separate matching standard. The deviations in stoichiometric coefficients were less than 6%.

3.1.3 Capillary and gel electrophoresis. There is relatively little interest in the possibilities for elemental speciation of *separation by GE coupled directly to the detector*, such as plasma source MS. This combination has been reviewed by Haider *et al.*[21] with the view to possibly changing this level of interest by highlighting its potential in proteomics, genomics and metallomics through discussion of several areas of application such as the determination of Au in nano-particles, Fe in metalloproteins, P in DNA, phosphoproteins, and phosphopeptides, I in aerosols, and the for study of cisplatin–oligonucleotide interactions. The *combination of PAGE and LA-ICP-MS* has been similarly examined[22] for its contribution to advances in metallomics (or more specifically, metalloproteomics). The reviewers focused on laser ablation as the interface between the separation and detection.

In the seventh biennial review article in a series examining the methodological developments in the *CE analysis of inorganic species*,[24] covering 198 publications between January 2009 and

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December 2010, (see also Section 1) the reviewers highlight, among other trends, the "constant pace of advances in speciation analysis". This is considered to be a separate category from applications to study the complexation and (bio)transformation reactions of metal analytes, which is labelled as "non-analytical" CE. Also highlighted are multi-detection designs, and ICP-MS interfaces. As a specific example of all of these topics, Ryvolova *et al.*[23] surveyed the aspects of metallothioniens (MT) that can be studied by CE with various detectors, including ICP-MS. It was pointed out that CE is an effective tool not only for the determination of MT in biological samples, but also for the identification of its isoforms and sub-isoforms. In addition, the reviewers consider that CE has a great potential for the investigation of metallothionein–metal and metallothionein–protein interactions, which has yet to be fully realized. In total, 186 references are cited to support the contention that CE has the potential to merge as a routine analytical technique for the continuing investigations of complex biological and biochemical processes involving metallothioneins.

The *interactions between OTC and human serum albumin* have been characterised by CE coupled with ICP-MS detection.[67] The coupling, to a MicroMist nebuliser, has been described previously,[68] and there would appear to be nothing new in the analytical instrumentation. The novelty is in the application of affinity CE and of nonequilibrium CE assays of equilibrium mixtures to the measurement of the binding constants of four OTCs (trimethyltin, tripropyltin, tributyltin, and triphenyltin) with human serum albumin. It was considered that the constants were determined with good precision and that the results confirm the suitability of CE-ICP-MS methods for further studies of organometallic complexation.

3.2 Chemical vapour generation

Several methods for *Hg speciation* have been developed, each based on a different principle. Angeli *et al.*[69] distinguished between iHg and organic Hg (MeHg and EtHg) by control of the tetrahydroborate concentration in a FI-AFS procedure. At very low values of the borohydride concentration (*e.g.* 10^{-5} M) and with the photoreactor turned off, only hydrides of the organic Hg compounds were produced, together with some mercury vapour. When the photoreactor (a microwave-powered mercury electrodeless discharge lamp) was turned on, all species were converted to Hg^{II} so that total Hg could be determined. The LOD for the method was 45 nmol L^{-1} (as Hg), which converts to the rather unimpressive 9 μg L^{-1}. The researchers investigated a recent claim by Wang *et al.*[70] that the addition of L-cysteine could weaken the Hg–C so that organo Hg compounds would react with BH without any prior digestion. However, this claim could not be corroborated, and the findings of Angeli *et al.* were described as being in "sharp contrast" to those of Wang *et al.* The method was validated by the determination of MeHg in TORT-1 (dogfish muscle) and applied to the analysis of 10 human saliva samples in which the total Hg concentrations of about 6 μg L^{-1} were less than the 12 μg L^{-1} in the blank. Despite what is implied in the abstract of the article, MeHg was not found in any of the samples, though spikes ranging from 60 to 200 μg L^{-1} were 85% recovered. Yin *et al.*[71] distinguished between the species by turning the flame in the AFS detector off, when only Hg vapour was measured. With the flame on, all volatile Hg species were detected including that produced from the reaction of MeHg with BH. The volatile species were generated a continuous flow manifold in which the acidified sample was merged with a stream of BH solution. There is no information about the flow rate of these two streams and the researchers did not, apparently, consider them to be parameters in the (single-cycle univariate) optimisation. The LODs were 0.03 μg L^{-1} and 0.05 μg L^{-1} for iHg and MeHg, respectively. The method was validated by the analysis of a CRM, TORT-2 (lobster hepatopancreas) and applied to the determination of the species, spiked at 2 μg L^{-1}, into three water samples. Zhang and Adeloju[42] exploited the effect of changing the BH concentration and the addition of a catalyst to the sample. After a thorough investigation of a large number of possible metal ion catalysts and the addition of thiourea to the BH reagent, conditions were found under which only iHg produced Hg vapour (0.0001% BH) and under which both iHg and MeHg gave the same sensitivity (0.1% BH with 100 mg L^{-1} Fe^{3+} added to the sample). The reactions were carried out in a merging zone FI manifold in which the sample was delivered at 9.6 mL min^{-1} and the reductant at 3.2 mL min^{-1}. When iHg was determined, the carrier stream was air, but was switched to dilute hydrochloric acid for the determination of MeHg. The method was validated by the analysis of SRM DOLT-4 (fish liver). The article contains a lengthy discussion of the possible catalytic mechanism and results to show that the addition of thiourea to the acid for extraction of the Hg species from the fish tissue considerably improves the extraction of iHg. The LOD was 0.2 μg L^{-1} using AAS for both species. Naozuka, and Nomura[72] developed a Hg speciation method based on selective thermochemistry inside a graphite furnace atomiser. Total Hg could be determined with solid sampling (powder and slurries were transferred into the furnace) when both a permanent iridium modifier was combined with palladium and magnesium added along with the sample. Under these conditions all Hg species were stabilised and sample matrix could be removed in the pyrolysis step. If tubes without the permanent modifier were used, then iHg was lost at about 120 °C, but MeHg was stable up to 280 °C. In the presence of sample matrix, these temperatures were shifted to 200 °C and 480 °C, respectively. The method was validated by the accurate analysis of CRMs CE-464 (tuna fish), TORT-2 (lobster hepatopancreas), and DOLT-2 (dogfish), and applied to the analysis of a candidate tuna fish reference material.

3.3 Developments in instrumentation

The *element speciation capabilities of current on-line aerosol MS* for both carbonaceous and non-carbon-containing aerosol species has been reviewed.[36] Essentially, there are two types of instrument: those with laser desorption/ionisation and those with thermal desorption electron impact ionisation. In the former, the particle sizing system triggers a high-energy laser pulse that instantly vaporises and ionises the particle in the ion source of the TOF mass spectrometer. Although this process is

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extremely efficient, enabling analysis of individual particles, it is susceptible to matrix effects that can significantly affect relative peak intensities within the mass spectrum. Therefore, typically "fingerprint" mass spectra are obtained that reflect the chemical type of the analysed aerosol. In the second type of spectrometer, multiple particles are flash-vaporised when hitting a hot (~600 °C) surface, and the evolved vapour is ionised by electron impact. This two-step process provides quantitative information about non-refractory particle components. However, because there is no separation step only limited information on individual species can be obtained. The review examines progress in the speciation of Cr, I, N and S and variety of C-containing particles. For N, S and C, it appears that considerable species information can be obtained; for example, nitrate, ammonium, amine and amino acid N can be distinguished. On the other hand, the information that can be obtained for Cr and I would appear to be much more limited and more strongly dependent on instrument operating conditions. The reviewer suggests that the co-location of both types of instruments would be helpful, so that the results of single particle analyses by laser desorption ionisation could be evaluated alongside the results obtained by the thermal desorption process (see also Section 1).

The group of Sanz-Medel[73] describe *an ICP-MS instrument containing a triple quadrupole mass analyser* as a new tool for absolute quantitative proteomics and phosphoproteomics. These areas of study need interference-free quantification at very low concentrations of ICP-detectable elements naturally present in proteins. The triple quad spectrometer allows a new way of removing polyatomic interferences in the determination of S (present in the majority of proteins as methionine or cysteine) and of P (present in phosphorylated proteins), based on the tandem mass spectrometry typically used in molecular MS. The first quadrupole was operated as band-pass mass filter (window 1 amu) to select target analyte ions (^{31}P, ^{32}S and their isobaric polyatomic interfering species). In the second quadrupole, only the ions selected react with oxygen, thereby decreasing both the interferences produced by matrix ions and the background noise. The product ions (PO^+ at m/z 47 and SO^+ at m/z 48) were separated in the third quadrupole and detected. Compared with the performance of a single quadrupole instrument with a dynamic reaction/collision cell, enhanced sensitivity and selectivity were obtained. The separation of S- and P-containing species by capillary HPLC followed by detection with the triple quad instrument was described. Limits of detection of 11 and 6.6 fmol, respectively, were obtained which, it was claimed, are the lowest ever published.

3.4 X-ray methods

Reports of elemental speciation in biological materials by X-ray methods remain relatively scarce. However, there has been a considerable increase in the numbers of reports of elemental speciation in solid inorganic materials during the current review period.

There are several reports of *studies of historical artifacts*. The main Fe pigments responsible for the dark-red coloration of Attic pottery fragments from the Gioiosa Guardia archaeological site in Sicily that date back to the VI–V centuries BC have been characterised. Absorption spectra were collected at the Fe K-edge on the Italian beamline for absorption and diffraction (BM8-GILDA) at the European Synchrotron Radiation Facility in Grenoble.[74] Analysis by both XANES and EXAFS showed that samples were a mixture of Fe_2O_3 (hematite or maghemite) and magnetite (Fe_3O_4), occurring in variety of relative abundances. Other researchers[75] at the Grenoble facility (on beam line BM-29), examined Co speciation in blue-and-white glazes from 16th to 17th century Chinese porcelains excavated in the Old-City part of Lisbon. Both the surface of the glazed debris and pressed pellets of slightly ground model compounds mixed with boron nitride were irradiated. Energy calibration was achieved by irradiating a cobalt metal foil and assigning the first inflection point to 7709 eV. The model compounds used were Co_3O_4, $CoAl_2O_4$ and Co_2SiO_4, plus a cobalt-based blue pigment (cerulean). The results confirmed a coordination environment of the pigmenting Co^{2+} ions close to tetrahedral and it was concluded that Co plays the dual roles of network former and modifier in these glazes. A third research group, working on beam lines ID21 and ID19, examined Mn speciation in 14th century stained glass windows originating from Sidney Sussex College, Cambridge,[76] in a study of the role of reducing reagents in the restoration of glass that is suffering from Mn-browning. Photoreduction observed with the focused (sub-μm) beam was avoided by defocusing the beam to about 10 μm in diameter. The XANES results clearly showed that treatment with hydroxylamine hydrochloride, which eliminates the dark coloration and restores the clear appearance of the glass, results in the reduction of Mn^{IV} to Mn^{II}. It was possible to map the distribution of species in the glass by μ-XRF by controlling the excitation energy. When the primary energy is 6.550 keV, Mn^{II} is excited and Mn^{IV} is somewhat excited, at higher energy (6.558 keV), Mn^{IV} is more efficiently excited, while at 6.587 keV, *i.e.*, well above the Mn–K edge, all species of Mn are excited with equal probability. By defining the sensitivity factors as the XANES intensity readings of the respective Mn-reference compounds, the fluorescent radiation intensity could be deconvoluted into the contributions from each species. Studies by high resolution computed tomography showed (a) the progress of the cleaning process, (b) that Mn was leached into the reducing solution, and (c) that the glass was, in fact, being damaged by the process.

Three *investigations of relevance to the nuclear industry using X-ray techniques* have been described. Measurements at the Swiss Light Source (SLS) in Villigen on both the μ-XAS beamline and the LUCIA beamline (for the Mg–K and Na–K μ-XRF maps) were used to study Ce speciation after long-term aqueous alteration (12 years at 90 °C and pH 9.6) of simulated nuclear waste glass.[77] The μ-XRF maps and μ-XANES spectra revealed that Ce was predominantly Ce^{IV} in the original glass, but that following dissolution from the glass matrix, was partly reduced and re-immobilised as Ce^{III} at grain boundaries or in the interstitial spaces between the glass particles. The concentration of Ce^{III} correlated with the spatial distribution of secondary Mg-clay formed as the main glass alteration product during the corrosion. It was argued that because redox-sensitive elements

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in the pristine glass (such as chromium, iron, and selenium) occur almost exclusively as oxidised species, reduction of Ce^{IV} was probably mediated by an external reductant, such as iron from the steel reaction vessel. The results showed that the glass was leached at relatively low oxidation potentials (E_h < 0.2 V) but a detailed examination of the equivalent E_h–pH diagram for plutonium revealed that under comparable conditions the reduction of Pu^{IV} to Pu^{III} would require considerably more reducing conditions (E_h < −0.3 V). It was concluded that Ce is not a good chemical analogue for plutonium, despite its wide use as a surrogate in simulated radioactive waste. Other workers studied Pu–U mixed oxide nuclear reactor fuels on the μ-XAS beam line (X05LA) at the same facility.[78] The size of the X-ray spot was about 2 μm and the energy calibration for both Pu and U was done in transmission mode using a thin UO_2 powder sample consisting of micro-particles of UO_2 diluted in a 3 mm boron nitride pellet. The centre and periphery of both fresh and irradiated rods were examined by μ-XRF, μ-XRD, as well as μ-XAFS, and it was concluded that even in the irradiated fuel, the Pu^{IV} is not oxidised to either Pu^{V} or Pu^{VI}. Frohlich *et al.*[79] point out that for certain European countries, such as France, Germany, and Switzerland, clay is considered to be a possible host 'rock' formation for the storage of high-level nuclear waste and that, in addition to other transuranium elements, ^{237}Np ($t_{1/2}$ 2.1 × 10^6 years) will contribute significantly to the radiotoxicity of this waste after storage times of more than a thousand years. It was argued that, over storage times of several hundred thousand years, escape of the radionuclides from their storage place is a possibility and interaction between these radioactive elements and the geological and technical barriers must be considered. Consequently, the uptake of Np species by Opalinus Clay (OPA) was studied using the facilities of both the Swiss Light Source and of the European Synchrotron Radiation Facility in Grenoble. Thin sections of OPA in contact with Np^{V} solution under aerobic and anaerobic conditions, as well as a diffusion sample, were analysed by μ-XRF to determine the elemental distributions of Ca, Fe and Np. Regions of high Np concentration were subsequently investigated by μXAFS to determine the oxidation state of Np, and information about the reactive crystalline mineral phases in the vicinity of Np enrichments was obtained from μ-XRD. They found that Np^{V} was reduced to Np^{IV} mostly under anaerobic conditions. Spatially resolved measurements showed that Np^{IV} tended to aggregate and that Np(V) was distributed more homogeneously on the OPA surface. It was concluded that in regard to the long-term storage of nuclear waste, these results support the suitability of this type of host rock because the highly mobile Np^{V} will be immobilised by reduction to Np^{IV} in the Opalinus Clay.

There have also been a number of *studies of particulate material*. The *Pb speciation in fly ash* has been studied by a combination of several methods including XAFS and XANES.[80] As the fly ash (collected during the processing of flue gases) from the incineration of municipal waste can contain relatively high concentrations (up to tens of thousands of mg kg^{-1}) of potentially hazardous elements, such as lead, assessing the success of any chemical treatment prior to disposal requires information about the lead species. Measurements were made at beam line 01B1 of the SPring-8 synchrotron radiation facility in Hyogo, Japan (one of only 4 facilities in the world with beam energy greater than 5 GeV). For the EXAFS experiments, the researchers prepared standards by mixing appropriate reagents with boron nitride, forming pellets, and storing in sealed polyethylene bags. The 13 fly ash samples were prepared by sealing the powder directly into polyethylene bags. It was found difficult to quantitatively examine the chemical species with EXAFS only; however, fitting to the XANES spectra enabled direct quantification of the chemical species, showing that $PbSiO_3$, $PbCl_2$, or $Pb_2O(OH)_2$ was the predominant species. Other elements were also determined by XRF spectrometry and ICP-OES and fractioned the lead (6 fractions) with a sequential extraction procedure. A somewhat similar approach was adopted by Shafer *et al.*[81] in a study of the *V speciation in particles emitted from diesel vehicles* and in urban atmospheric aerosols. A four-stage fractionation of particle-size resolved material was coupled with an SPE procedures of the separation of V^{IV} and V^{V} with quantification by ICP-MS. Particles containing V were identified, at the microprobe beamline 10.3.2 (Advanced Light Source, Lawrence Berkeley National Laboratory, Berkeley, CA), by XRF mapping using a focused beam (5 μm^2 spot on the sample). The XANES 1s spectra, collected over the energy range 5350–5720 eV, were compared with a spectral library of 19 vanadium-bearing materials. Principal component analysis, target transformation analysis, and linear least-squares fitting with ALS BL10.3.2 custom and SixPack software identified V_2O_5 (considered particularly toxic with mutagenic effects, respiratory tract toxicity, and possible carcinogenic activity) as the dominant species in the fine particles emitted by a selective catalytic reductor. Particulates forming in the expanding plume from a hydrothermal vent were collected[82] with a new sampling tool, the suspended particulate rosette sampler, and analysed by a variety of techniques including scanning transmission X-ray microscopy, XRF mapping, S 1s and Fe 1s μXANES, and μXRD at beamline 10.3.2 of the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory. Spectra were calibrated using the inflection point of elemental S at 2472 eV and of Fe foil at 7110.75 eV. Principal component analysis (PCA) and target transformation analysis were performed on an Fe XANES dataset (26 spectra; 7010–7410 eV range) to determine the number of species present and evaluate the suitability of standards, using SixPack software. Sulfur XANES data (2420–2575 eV range) were compared with reference spectra. The researchers found over 20 different minerals, nine of which were either not predicted by thermodynamic modelling or had no close match in their thermodynamic database. The S-bearing phases consisted of polysulfides (S-6, S-8), and metal sulfides (Fe, Cu, Zn, Mn) and four dominant species, Fe oxyhydroxide, Fe monosulfide, pyrrhotite, and pyrite, accounted for over 80% of the Fe present. Arsenic was associated with both Fe oxyhydroxides and sulfides but not uniformly distributed among either mineral type.

With regard to *applications to biological systems*, the contribution of X-ray techniques to the speciation and location of As, Ge and Se in rice has been reviewed[35] as was discussed earlier in Section 1. The chemical composition and S speciation in chick

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corneas at several stages of embryonic development were studied[83] by several techniques at beamline ID 21 of the European Synchrotron Radiation Facility. The S-containing compounds were identified by comparing the absolute energy values to the respective energies for standard S compounds obtained from previous studies. It was pointed out that it is not possible to distinguish between (a) inorganic sulfate (SO_4^{2-}) and ester sulfate (R–O–SO_3), and (b) thiols (R–SH) and organic monosulfides (R–S–R′) because their XANES spectra peak energies are identical. Nonetheless, the researchers considered they could discern developmentally regulated alterations in thiols, organic monosulfides, ester sulfate, and inorganic sulfate species. It was also pointed out that the experimental and analytical approach documented was new and potentially has wide applicability in the life sciences. Studies of Cd species in *Arabidopsis halleri*, a model Cd and zinc hyperaccumulator,[84] were also performed at the European Synchrotron Radiation Facility in Grenoble (beamline FAME, BM30B) where Cd K-edge bulk EXAFS spectra for the plant leaves and reference compounds were recorded. It was found that Cd was predominantly bound the COOH/OH groups in organic acids and/or cell wall components with less than 25% of the Cd bound to thiols, which could be glutathione (found in significant amounts), but phytochelatins were not detected. It was concluded that the mechanisms of Cd storage and detoxification in *A. halleri* differ from those previously found for zinc. The introduction to the article contains many references to previous work in this area. Similar results were reported for the accumulation of Cd by the willow *Salix miyabeana*[85] based on studies performed on beam line 37XU of the SPring-8 synchrotron facility in Hyogo, Japan. Application of μ-XRF and μ-XANES to the two-dimensional Cd distribution in segments of young stems, obtained with approximately 2 μm^2 beams, showed the predominant localisation of Cd in the apoplastic region (the free diffusional space outside the plasma membrane) and that the apoplastic detoxification of Cd depends on Cd–oxygen, but not on Cd–sulfur, interactions. Researchers have reported (in Chinese) on a study of Pb speciation in duckweed by XANES.[86] The abstract of the article does not indicate where the experiments were performed. The duckweed, which can tolerate and accumulate, "heavy" metals, came from an area impacted by the Qixiashan lead–zinc mine of Nanjing and contained 39 mg kg^{-1} Pb. The XANES spectra showed that all of the Pb was accounted for as stearate (65%) and sulfide (37%), which was interpreted as a sulfur-containing organic acid species. An investigation of Cu and Zn distribution, speciation and potential bioavailability from treated sewage sludge applied to agricultural land has been reported.[87] This work involved the use of a number of techniques. The K-edge EXAFS spectra were collected at room temperature in the fluorescence mode on beamline BL20-B at the Photon Factory, Tsukuba, Japan. Elemental μ-XRF mapping was conducted at the Australian Synchrotron X-ray Fluorescence Microscopy beamline. Mapped samples collected on polyimide tape squares were leached with 0.7 M NaOCl adjusted to pH 8.5 at 90 °C for 2 h. After rinsing, the samples were remounted for repeat μ-XRF mapping of the same sample areas. The researchers concluded that Cu speciation was consistently dominated by sorption to organic matter, whereas Zn partitioned mainly to iron oxides and, therefore, further risk assessment studies should focus on Cu as it is associated with the mineralisable fraction.

A study of *chlorine speciation in particulate matter* by wavelength-dispersive PIXE spectroscopy may also be of interest,[88] Researchers were able to distinguish between chloride from sea salt and other sources at concentrations down to 1% in the particles. The *investigation of the influence of U speciation* on its accumulation and translocation in three plant species: oilseed rape, sunflower and wheat has been reported,[89] It was found that complexation with carbonate or citrate decreases the U content in roots but increases translocation to the shoots considerably, resulting in accumulation in the leaves. However, complexation with phosphate considerably reduced accumulation in all tissues. It was also suggested that there was evidence for U-binding proteins in plant tissues. The *detection of titanium dioxide particles in frozen tissue* sections using synchrotron XRF has been studied.[90] The focus of this work concentrated on the 5 μm titanium dioxide particles that are widely used in cosmetics in response to general concerns about the safety of such nanoparticulate matter.

4 CRMs and metrology

A *new Hijiki certified reference material, NMIJ CRM 7405-a*,[91] has been produced this year with certified values for 18 elements, including total As, and for water soluble As^V at 35.8 ± 0.9 and 10.1 ± 0.5 ($k = 2$) mg kg^{-1} respectively. It should be noted that an Erratum paper has subsequently been published for this work.[92] The As^V in the Hijiki powder was extracted by both UAE and MAE with 0.5 g of sample suspended in 20 g water in each case. Sonication was for one hour for UAE whilst the MAE conditions were: ramp to 60 °C over 5 minutes, hold at 60 °C for 30 minutes and cooling to RT over 10 minutes. In each case the extract solutions were centrifuged at 400 rpm for 10 minutes followed by filtration through a 0.45 μm PVDF filter with, AC added as an internal standard to the filtrate. The remaining Hijiki powder residue was then subjected to further high temperature MAE to allow a mass balance to be calculated. Subsequently, the As^V mass fraction in the extracts was determined by HPLC-ICP-MS, using a C18 column (4.6 mm × 150 mm) with the mobile phase containing 10 mmol L^{-1} sodium butanesulfonate, 4 mmol L^{-1} TMAH, 4 mmol L^{-1} malonic acid and 0.05% (v/v) methanol, adjusted to a pH of 3.0. In addition the MAE extracts were also analysed by AEC-IPC-MS, PRP X-100 column (4.1 mm × 150 mm) with the mobile phase comprising 20 mmol L^{-1} $NH_4H_2PO_4$ adjusted to pH 5.6. For these analyses He, at 2.0 mL min^{-1}, was used as a collision cell gas to negate potential polyatomic interferences on ^{75}As. Insufficient As^{III} was detected in the Hijiki extracts to allow for reliable certification of this species.

A *sewage sludge QC material, with DBT and TBT as the target analytes*, has been produced for future use in an intercomparison study.[93] Three separate extraction procedures, (a) MAE (ramp to 50 °C in one minute, hold for three minutes), (b) UAE for 30 minutes and (c) mechanical stirring for 16 hours, were employed with 0.2 g of sample being suspended in 4 mL of

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glacial acetic acid in each case. All extracts were then derivatised, after centrifugation, with $NaBEt_4$ before extraction into isooctane prior to analysis by either GC-MS or ID-GC-ICP-MS. For the latter a ^{119}Sn enriched DBT and TBT spike was added prior to species extraction and the sample shaken for only 15 minutes to achieve equilibration. The values found for each extraction/analytical technique combination were not statistically different leading to reference values of 1533 ± 77 ng g^{-1} for DBT and 534 ± 37 ng g^{-1} for TBT with the stated combined uncertainties having a coverage factor of 2.

The determination of SeMet in human serum by IDMS has received further attention in the period under review. One study focused on the evaluation of and sources of uncertainty in, three reference materials: BCR-637 and Seronorm L1 and L2. These materials, which have certified and indicative values for total Se respectively, were examined by SSIDMS.[94] The SeMet was extracted from 0.45 g serum, after the addition of a ^{76}SeMet, with 6 mol L^{-1} urea and 0.1 mol L^{-1} dithiothreitol with mechanical shaking for 1 h. Following the addition of a mixture of protease (15 mg mL^{-1}) and lipase (8 mg mL^{-1}) the mixture was shaken for a further 15 min and incubated at 37 °C for 17 h in order to complete the enzymatic reaction. Finally, 20 μL of glacial acetic acid was added and the resulting solution stored at −20 °C until analysis. The amount of added spike was optimised to give a $^{80}Se : ^{76}Se$ ratio of close to unity and a $^{78}Se : ^{76}Se$ ratio close to 0.5. Separation was achieved using HPLC (C18, 15 cm × 4.6 mm id × 4 μm, with a mobile phase of 0.1% pentafluorpropionic acid in 2% MeOH) followed by detection using an ICP-MS instrument utilising He (2.5 mL min^{-1}) and H_2 (4 mL min^{-1}) as collision/reaction gases to determine the Se ratios. The values found, expanded uncertainties and uncertainty sources identified were reported to be highly comparable for the calculated mass fraction of SeMet in each sample regardless of which isotope pair was used for the calculations. The major uncertainty contributions arose from the mass fraction of the spike and mass bias correction factor (determined by sample bracketing) which, when combined, contributed between 90 and 95% of the expanded uncertainty. The remaining uncertainty arose from the repeatability of five individual replicate samples and the measured isotope amount ratios. It was concluded that the found mass fractions of SeMet in the three serum standards analysed could be used as indicative values for method validation for the determination of SeMet in human serum. The second paper 33/1042 investigated the impact of pump flow fluctuations, for the added post-column spike, for SUIDMS. The enzymatic digestion procedure used to extract SeMet from the serum is only briefly described. The HPLC separation involved the use of a C8 column (5 μm, 3 mm i.d. × 250 mm) with an eluent of 0.1% TFA in 2% MeOH which was coupled to an ICP-MS instrument using 2.7 mL min^{-1} H_2 and 2 mL min^{-1} He as reaction/collision gases. The spike mass flow was monitored gravimetrically at a frequency of 0.5 Hz. The data was then compared with data for the same sample for SeMet by SSIDMS. The results of both approaches were in agreement with better agreement found when the SUIDMS value was corrected for the time lag in the measured spike flow rate and the actual mixing of the sample and spike flows.

5 Elemental speciation analysis

5.1 Antimony

Few studies have looked at the speciation of Sb during this review period. *Drugs based on Sb^V* are first-line treatment of the parasite disease leishmaniasis. It is generally believed that Sb^V acts as a prodrug, which is activated by reduction to Sb^{III}; however, the site of reduction is not known. It has been hypothesised that the reduction takes place in the parasites' host cells, the macrophages. In a study by Hansen *et al.*,[95] the human macrophage cell line Mono Mac 6 was exposed to Sb^V in form of the drug sodium stibogluconate (Pentostam™). Cell extracts were analysed for Sb species by HPLC-ICP-MS, and it was found that Sb^V is actually reduced to Sb^{III} in the macrophages; up to 23% of the intracellular Sb was found as Sb^{III}. Transfer of the cells to an Sb-free medium rapidly decreased their Sb^V and Sb^{III} content. Induction of the cell's production of reactive oxygen species did not have any marked effect on the intracellular amounts of Sb^{III}.

The development of treatment methods to reduce *Sb leaching from municipal solid waste* incinerator bottom ash, such as accelerated carbonation, is being complicated by insufficient understanding of Sb geochemistry. The leaching of Sb^V and Sb^{III} in Municipal Solid Waste Incinerator bottom ash was studied as a function of pH and degree of carbonation.[96] While total (Sb^V + Sb^{III}) leaching was lowest (1.2 mg kg^{-1}) at the natural pH of uncarbonated bottom ash (*i.e.* 10.6), HPLC-ICP-MS analysis showed that acidification and carbonation increased Sb^V leaching, but decreased Sb^{III} leaching, probably because $Sb^{III}(OH)_4^-$ became less stable. Geochemical modelling, using PHREEQC software, suggested that Sb^V concentrations approached equilibrium with the romeites, *i.e.* Ca antimonates, at pH = 10.6 and $Ca[Sb(OH)_6]^2$ at pH = 8. It was hypothesised that dissolution of romeite controls antimonate leaching in the pH range 8–11 in bottom ash, because while Ca is preferentially leached from romeite, the mineral structures containing more Ca at higher pH are less soluble. A model is proposed where acidification and carbonation both lead to lower Ca^{2+} and/or hydroxyl concentration, which removes Ca^{2+} and hydroxyls from the romeite structure and leads to comparably higher Sb^V concentration in equilibrium with romeite. Antimony solubility depends on pH and Ca^{2+} availability in this model, which has implications for bottom ash valorisation and risk assessment.

5.2 Arsenic

Most As speciation papers published within the review period have focused on specific applications rather than the development of new methodologies. Arsenic speciation determined in environmental samples by HG and ET-AAS has been reviewed by Anawar.[97] The review included recent improvements and modifications in separation techniques and stressed the use of L-cysteine as pre-reductant over KI to reduce the interference of transition metals on the arsine generation, although most techniques covered were well established. A solid phase preconcentration procedure using hybrid sorbent based on nano zirconium dioxide-boron oxide for the speciation and

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determination of As^{III}, As^{V} and total As in water samples by HG-AAS has been reported.[98] The adsorption capacity was 98.04 mg g^{-1} for As^{V} and the hybrid sorbent was stable for up to 100 runs, offering recoveries of 99 ± 5% with a LOD of 9.25 ng L^{-1}. The accuracy of the method was tested by analysing a CRM (SPS-WW1 Waste Water) and spiked real samples. The separation and determination of As species in water by selective exchange and hybrid resins has also been reported.[99] Three types of resins: a strong base anion exchange (SBAE) and two hybrid (HY) resins: HY-Fe and HY-AgCl, based on the activity of hydrated iron oxides and a silver chloride were investigated. It was found that the sorption processes (ion exchange, adsorption and chemisorptions) of As species on SBAE (ion exchange) and HY resins depended on the pH values of the water samples. This was exploited to determine the different species present. Both ICP-MS and HG-AAS were employed as detectors, ICP-MS offering a LOD of 0.2 μg L^{-1} and RSD values between 3.5 and 5.1%. A little more unusually, Matusiewicz and Slachcinski[100] have reported a novel hyphenated technique, a microfluidic chip-based CE-HG system interfaced with a MIP–OES, to provide two iAs species separation capabilities. A MiraMist CE nebuliser was used as the μchip-CE-HG-MIP interface. To facilitate the chip-CE effluent delivery and to provide the necessary medium for subsequent volatile species generation, diluted HCl solution was introduced on the chip. Inorganic As^{III} and As^{V} were chosen as the targets to demonstrate the performance of the technique. Baseline separation of As^{III} and As^{V} was achieved within 70 s in a 26 mm long channel at 2.8 kV using a mixture of borate buffer and cetyl trimethylammonium bromide (CTAB) (pH 9.5) as an electrolyte. The RSDs of peak height, based on six determinations of 50 ng mL^{-1} standard of As^{III} and As^{V}, were 5% and 7%, respectively. The detection limits (3 sigma) based on peak height measurement were 3.9 ng mL^{-1} and 5.4 ng mL^{-1} for As^{III} and As^{V}, respectively. The kinetics and efficiencies of As^{III} and As^{V} removal from water were evaluated using poly-aluminium granulates (PAG) with high content of aluminium nanoclusters.[101] The PAG was characterised to be meso- and macroporous, with a specific surface area of 35 ± 1 m^2 g^{-1}. Adsorption experiments were conducted at pH 7.5 in deionised water and synthetic water with the composition of As-contaminated groundwater. The As^{III} and As^{V} sorption was best described by the Freundlich and Langmuir isotherms, respectively, with a maximum As^{V} uptake capacity of 200 μmol g^{-1} in synthetic water. While As^{III} removal reached equilibrium within 40 h, As^{V} was removed almost entirely within 20 h. Electron microscopy and μXRF revealed that As^{III} was distributed uniformly within the grain, whereas As^{V} diffused up to 81 pm into PAG. The results imply that As^{V} is adsorbed 3 times faster while being transported 105 times slower than As^{III} in Al hydroxide materials.

The use of *cellulose fibres coated* with an yttrium hydroxide layer has been proposed as a novel green sorbent using no organic solvents.[102] A mini-column was incorporated into a sequential injection system for selective uptake of As^{V} at pH 11.5 which was later recovered with 50 μL of 0.8 mol L^{-1} NaOH solution as eluent, pre-reduction by KI-ascorbic acid (5%, W/V), and detection by HG-AFS. Total iAs was quantitatively taken up at pH 6.0 by following the same procedure and As speciation was performed by difference. With a sample volume of 1.0 mL, an enrichment factor of 16.4 was derived with a LOD of 17 ng L^{-1}. The RSD was 2.6% (0.5 μg L^{-1}, $n = 11$). The procedure was validated using CRM GBW 09101 (human hair). In previous publications using ferrihydrite-backed diffusive gradients in thin films (DGT) devices to determine As, the organic forms have often been disregarded, even though it is known that the two most prevalent in natural waters, DMA and MMA, may adsorb to ferrihydrite and thereby be included in the measurement. Osterlund *et al.*[103] have demonstrated that MMA, and under acidic conditions DMA, adsorbed to the binding layer and might therefore contribute to the total mass of measured As. Diffusion coefficients were measured for all four species to enable quantification of DGT-labile concentrations of organic and iAs. Elution of the analytes from the ferrihydrite binding layer was performed using 1 mL of 1 M NaOH to facilitate As speciation analysis using chromatographic separation. Average recovery rates were between 87 and 108%. This study shows that the contribution of DMA and MMA to the total accumulated mass must be taken into consideration when evaluating DGT data in future studies. A multisyringe flow-injection approach has also been coupled to HG-AFS with UV photo-oxidation for DMA, iAs and total As determination, depending on the pre-treatment given to the sample (extraction or digestion).[104] The LOD's for DMA and iAs were 0.09 and 0.47 μg L^{-1}, respectively. The method was validated by means of a CRM, BCR-627 (muscle of tuna) with good agreement with the certified values.

Accurate *measurement of As in air* is critical to providing a more robust understanding of arsenic exposures and associated human health risks. Although there is extensive information available on total As in air, less is known on the relative contribution of each As species. Several studies have been reported to address this. Lewis *et al.*[105] have reviewed measurement methodology (including ICP-MS, HG-AAS and HG-QF-AAS) and risk assessment considerations and have highlighted that despite differences in seasonal factors, study duration, sample collection methods, and analytical methods, there is adequate data to show that As in air is mainly in the inorganic form. Reported average concentrations of As^{III} and As^{V} ranged up to 7.4 and 10.4 ng m^{-3} respectively, with As^{V} being more prevalent than As^{III} in most studies. Concentrations of the organic As compounds are usually negligible (in the pg m^{-3} range). The authors were unable to determine the variation in arsenic composition as a function of source or PM fraction. The health implications of the distribution of As species in airborne particulate matter at an urban site (Huelva) in SW Spain has been studied.[106] Samples were taken between 2001 and 2008 in an area adjacent to a Cu smelter and the results obtained by ICP-MS showed that As accumulates preferentially (*ca.* 70–80%) in the particles with smaller diameter (PM2.5 *versus* PM10), representing a threat to human health due to the higher capacity of the finer particles to enter an organism through the respiratory system. Speciation analysis performed with HPLC-HG-AFS showed that As^{V} represented the main species, but As^{III} was also found at significant concentration, representing a 5–10% of the total As content. The results also

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indicate that As^{III} tends to concentrate preferentially in the finer fraction PM2.5 in comparison with As^{V}, thus representing an added health risk for the local population. In a similar study[107] HG-QT-AAS was used for the determination of As(III) and As(V) associated with total suspended particles (TSP) and PM10 particles collected from urban and rural atmosphere in Iran. It was suggested that the good co-variation in concentrations of As, PM10 and CO indicated that As is mostly released into the atmosphere from anthropogenic sources and especially from combustion of fossil fuels. Yang *et al.*[108] have reported on the speciation of As in the atmosphere in Beijing, China from February 2009 to March 2011. HPLC-HG-AFS was used and the total As concentrations ranged from 0.03 to 0.31 $\mu g\ m^{-3}$ (mean: $0.13 \pm 0.06\ \mu g\ m^{-3}$) in Beijing's air. The concentrations of As^{III} and As^{V} ranged from 0.73 to 20 $ng\ m^{-3}$ (mean: $4.7 \pm 3.6\ ng\ m^{-3}$) and from 14 to $2.5 \times 10^2\ ng\ m^{-3}$ (mean: $67 \pm 35\ ng\ m^{-3}$), respectively. The As levels and speciation demonstrated relative higher levels in spring and autumn and lower values in summer and winter. Of the extractable species in the total suspended particle samples As^{V} accounted for 81 to 99%. Organoarsenic species, MMA and DMA were not found in all samples. Direct characterisation of airborne particles associated with As-rich mine tailings, including particle size, mineralogy and texture, have been studied around an abandoned Au mine tailings from Nova Scotia, Canada.[109] Total concentrations of As in the <8 μm fraction varied from 65 to 1040 $ng\ m^3$ of air as measured by PIXE analysis. The same samples were analysed by μXANES and μXRD and found to contain multiple As-bearing mineral species, including Fe–As weathering products. The As species present in the dust were similar to those observed in the near-surface tailings.

Arsenic in drinking water remains a concern in many parts of the world, and once again there have been numerous studies in this area. In Pakistan, a study evaluated and interpreted data sets from water samples collected from different sampling origins of ground water (hand pump and tube well) and surface water (municipal, river and canal) using PCA.[110] The aim was to provide information concerning potential pollution sources to obtain better information about water quality. In this case the As^{III} was complexed with APDC and extracted in Triton X-114, while total was adsorbed on titanium dioxide and determined by ET-AAS. Recovery was >98% and the accuracy was confirmed by standard addition. The mean concentration of As^{III} and As^{V} in the surface water samples was found to be 15.8 and 6.00 $\mu g\ L^{-1}$, respectively, and in the ground water samples As^{III} and As^{V} ranged from 6.20 to 51.0 and 6.40 to 53.0 $\mu g\ L^{-1}$, respectively. The As exposure from drinking water on pregnant women and their children was studied In rural Bangladesh.[111] Mother child pairs ($n = 1951$) were followed from 2001 to 2003. Urine As was measured by HPLC-HG-ICP-MS. The children had elevated urine-As at 5 years old (median = 51 $\mu g\ L^{-1}$), and the urine-As distribution was similar to that observed in the mothers during gestation. Children's U-As at 5 years old correlated with their urine-As at 1.5 years old and to maternal urine-As during early and late gestation.

Arsenic speciation in freshwater systems continues to be a popular subject for study. The two complementary techniques, HPLC-ICP-MS and XANES were used to assess As speciation in freshwater phytoplankton and zooplankton collected from As-contaminated lakes in Yellowknife, Canada.[112] Arsenic concentrations found in the lake water ranged from 7 $\mu g\ L^{-1}$ in a non-contaminated lake to 250 $\mu g\ L^{-1}$ in mine-contaminated lakes, which resulted in As concentrations ranging from 7 to 340 $mg\ kg^{-1}$ (dry wt) in zooplankton organisms and from 154 to 894 $mg\ kg^{-1}$ (dry wt) in phytoplankton. The main As compounds identified by were iAs (from 38% to 98% of total As). No other As compounds were found in phytoplankton, but zooplankton organisms showed the presence of organoarsenic compounds, the most common being the sulfate arsenosugar, up to 47% of total As, with traces of phosphate sugar, glycerol sugar, MMA, and DMA. The same group also reported on the zooplankton species *Daphnia pulex* collected from a lake situated on As-rich tailings from a historical gold mine in the same area.[113] The same techniques were used and the lake water was found to contain 0.25 $mg\ L^{-1}$, while the zooplankton organisms contained up to 35 $mg\ kg^{-1}$ As. The *D. pulex* mainly contain iAs with 56% of As as As^{V} and 10% as As^{III}. The remaining 34% were identified as MMA, DMA, and arsenosugars. Arsenic immobilisation by an Fe^{II}-oxidising freshwater bacteria has been investigated using a STXM.[114] The workers looked at of the spatial distribution of As^{V} within cell-mineral aggregates formed by *Acidovorax sp.* strain BoFeN1, an anaerobic nitrate-reducing Fe^{II}-oxidising beta-proteobacteria isolated from the sediments of Lake Constance. This species is noted to be highly tolerant to high levels of As^{V}. Related, As-tolerant Acidovorax-strains have been found in As-contaminated groundwater wells in Bangladesh and Cambodia wherein they might influence the mobility of As by providing sorption sites which might have different properties as compared to chemically formed Fe-minerals.

Several research groups have used *mice as potential bio-indicators* for environmental pollution, such as the free-living mouse *Mus spretus* found in different areas of the Doana National Park in SW Spain.[115] An instrumental coupling of SEC-UV-ICP-MS was used in both analytical and preparative scale to identify the As species in cytosolic extracts from high metabolic activity organs kidneys, liver, and brain. The results showed a higher presence of MMA and DMA in kidneys of mice caught in contaminated areas and the existence of small amounts of unidentified arsenicals when CEC was used, which could be related to the presence of dimethylarsinoylethanol (DMAE), thioarsenic species, or AC. Dose-dependent urinary phenotype of iAs methylation in mice with a focus on As^{III} methylated metabolites has also been reported.[116] The goal was to investigate the As metabolites in relation to As^{III} dose *via* immediate analysis of fresh urine samples, while preventing the oxidation of unstable methylated As^{III}-containing metabolites. Female mice (C57BL/6) received sodium arsenite by gavage at doses of 0, 3, 6 or 10 mg As kg^{-1} per day for 9 days, after which trivalent methylated arsenicals were detected in 100% of urine samples; these arsenicals were not detected in the urine of control mice. The amount of DMA^{III} detected in urine depended on the dose of As^{III} administered and was determined to be 50.2%, 31.4% and 16.5% of the total urinary As in mice exposed to 3, 6, or 10

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mg As kg^{-1} per day, respectively. This relationship is consistent with the hypothesis of inhibition or saturation of iAs methylation. Urinary methylation profiles were also investigated in female C57BL/6J black mice given drinking water containing 500, 250 0r 100 μg As^V L^{-1}, as sodium arsenate for 2 months.[117] The concentrations of As chosen reflected those in the drinking water often encountered in As-endemic areas. Urine samples were collected from the mice at the end of the exposure period, and the As species were analysed by HPLC-ICP-MS. All detectable As species showed strong linear correlation with the administered dosage. The methylation patterns were similar in all three groups with a slight decrease of the DMA : As^V ratio in the 500 μg L^{-1} group, which corresponded to the significantly higher As retention in the tissue. The results indicate that urinary As could be used as a good biomarker for internal dose and potential biological effects.

Arsenic accumulation and speciation in plants from different habitats has been studied by Bergqvist and Greger[118] in order to find suitable candidates for phytoremediation purposes and to investigate specific accumulation patterns due to growth habitat and plant groups. The As accumulation in 124 plant species collected from different habitats and speciation in 6 of these plant species was determined using HPLC-FAAS (Hamilton PRP X-100 column (250 mm × 4.6 mm) with ammonium-phosphate buffer at pH 5.8). The data show that submerged plants have a higher accumulation than emergent and terrestrial plants. The As concentration in terrestrial and emergent plants were correlated with the [As](soil), while the accumulation factor correlated negatively with [As](soil). Gymnosperms had a high [As](shoot) : [As](root) ratio. The iAs species, arsenate and arsenite were found in plants from all habitats and MMA in all but one plant species. Arsenate predominated in submerged plants. The results suggest that the habitat and the [As](soil) have a strong influence on the As accumulation in plants and that submerged plants and/or gymnosperms might be suitable for phytoremediation of As. An evaluation of As speciation and bioaccumulation in different plant species from two highly polluted mining areas in N. Spain has been reported.[119] Accumulation rates depend on both the level of pollution of the site and on the plant species itself. Total As concentrations also varied depending on the part of the plant, with roots accumulating the most As in all the studied plants (up to 1400 mg kg^{-1}). Extraction efficiencies (using 0.3 M orthophosphoric acid) were above 80% and speciation was not altered, since recoveries of the spiked As species (As^{III}, As^V, DMA and MMA) were between 94 and 102%. Speciation studies were performed using HPLC-ICP-MS and showed As^V was always the predominant species, although significant levels of As^{III} was found for all samples. Specific plants, such as the desert plant *Prosopis juliflora-velutina*, have been studied.[120] Changes in As speciation induced by the rhizosphere were monitored using μSR-XRF combined with μXANES. The plants were grown in a sandy clay loam treated with As^{III} and As^V at 40 mg kg^{-1}. The results showed that As^V was the predominant species in the soil (rhizosphere and non-vegetated), whereas As^{III} was dominant in the root tissues from both As^V and As^{III} treated plants. Studies of thin sections from resin embedded soil cores revealed As^{III}–S interactions in root tissues and predominant As–Fe interaction in the soil. A non-chromatographic speciation method has been developed for the screening of iAs in garlic samples (*Allium sativum* L.) by HG-AFS using differential reduction.[121] The methodology offered an LOD of 0.8 and 0.6 ng g^{-1} for As^{III} and As^V, respectively with RSDs of 4% for As^{III} and 7% for As^V. The As^{III} content varied from 17.1 to 22.1 ng g^{-1} and As^V from 54.7 to 67.6 ng g^{-1}. The accuracy of the method was confirmed using SRM 1573a tomato leaves treated in the same way as the garlic samples. The distribution of As in hydrated and fresh cowpea roots (*Vigna unguiculata*) has been determined using SR-XRF (including sequential computed tomography).[122] The seedlings were exposed to 4 or 20 μM As^V or 4 or 20 μM As^{III}. For plants exposed to As^V, the highest concentrations were observed internally at the root apex (meristem), with As also accumulating in the root border cells and at the endodermis. When exposed to As^{III}, the endodermis was again a site of accumulation, although no As was observed in border cells. For As^V, subsequent transfer of seedlings to an As-free solution resulted in a decrease in tissue As concentrations, but growth did not improve. These data suggested that, under the experimental conditions used, the accumulation of As caused permanent damage to the meristem.

The *speciation of As in food stuffs* continue to be a subject of particular interest. Various authors have reported on As speciation in seafood. A continuous leaching method coupled online with ICP-MS detection has been used to assess the maximum bioaccessibility of As in seafood samples.[123] The method simulated continuous-flow digestion by successively pumping artificial saliva, gastric and intestinal juices through a mini-column of powdered sample directly connected to the nebuliser of an ICP-MS instrument. The method allowed the real-time measurement of As being released by a given reagent. Because the analyte is continuously removed from the system, in contrast to batch methods, the dissolution equilibrium is driven to the right, hence quickly providing information about the worst-case scenario. Following consecutive leaching by the digestive reagents, the leachates were subject to speciation analysis by IC-ICP-MS to determine the As species released. Finally, the remaining residue from the mini-column was fully digested to verify mass balance. The method was used to determine the bioaccessibility of total As and As species in four CRM's and in several real seafood samples. The mass balance was verified in each case. Generally speaking, the non-toxic form was easily released whereas the inorganic forms were poorly bioaccessible. The separation of As^{III}, As^V, MMA, DMA, AsB, arsenosugar PO_4, arsenosugar OH, arsenosugar SO_3 and arsenosugar SO_4 were carried out to determine As speciation in sea fish and shellfish.[124] A Hamilton PRP-X100 anion-exchange column with a mobile phase of 20 mmol L^{-1} NH_4HCO_3 (pH 10.3) and a Dionex Ionpac CS-10 cation-exchange column with a mobile phase of 5 mmol L^{-1} pyridinium (pH 2.0) was coupled to ICP-MS to achieve this. The results showed that main components in all of the extracted fish and shellfish samples were AsB (42.8% to 99.4%), while arsenosugar PO_4 and arsenosugar OH were detected in most extracted shellfish samples (32.5% to 57.1%, 1.4% to 3.3% respectively). In a few extracted samples

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DMA was detected (5.7% to 25.0%). In contrast, no iAs was detected. The generic need for CRMs when determining iAs in seafood has been discussed by Petursdottir *et al.*[125] Utilising an alkaline-ethanolic solution and microwave heating, the CRM TORT-2 was subjected to different instrumental methodologies HPLC-ICP-MS; HG-ICP-MS and HPLC-HG-AFS. The methods gave a value of iAs, which is near the mean value of the reported values in the literature, but which ranged by a factor of 10. Inorganic As, that is, the sum of As^{III} and As^{V} and any thiol-bound As, was found to be less than 4% of the total As in 12 samples of fish meal when subjected to this extraction method followed by HPLC-ICP-MS. To date, there is no certified value of iAs in a seafood-based CRM to compare to in order to validate the findings. This illustrates the difficulties in quantitative determination of iAs in seafood and the need for a CRM for iAs and proficiency tests in order to introduce legislation for a maximum level of iAs in seafood and feed. The bioavailability of total As, As^{III}, As^{V}, MMA, DMA, AB, and AC has been assessed in different raw seafood samples (white fish, cold water fish and molluscs) by using an *in vitro* model that combines simulated gastric and intestinal digestion/dialysis methods.[126] Correlations between As species bioaccessibility and seafood nutrient contents (fat and protein) were also established. Total As content in seafood samples, and dialysable and non-dialysable fractions, were analysed by ICP-MS after an microwave-assisted acid digestion treatment. The As species, after matrix solid phase dispersion, and the species in the dialysable fraction were determined by HPLC-ICP-MS. Accuracy was assessed by analysing DORM-2 and BCR-627 CRMs and a mass-balance study. High dialysability percentages for total As and for As species were found (*i.e.* from 84.6 ± 1.7% to 106 ± 2.6%), and the bioaccessibility of As exhibited a negative correlation with the fat content of the seafood. However, no correlation was observed between the bioaccessible fraction of total As and As species and the protein content of the seafood studied.

Francesconi's group continue to report on *new arsenolipids*. In a recent publication,[127] 11 new compounds, were identified and quantified in two species of brown algae, Wakame (*Undaria pinnatifida*) and Hijiki (*Hizikia fusiformis*), by high resolution MS, HPLC-MS and GC-MS. Both algal species contained arsenosugar-phospholipids as the major type of arsenolipid, and arsenic-hydrocarbons were also significant components, particularly in Hijiki. The same group have presented a screening method for lipid-soluble As compounds (arsenolipids) in fish oils by reversed-phase HPLC-ICP-MS using a gradient elution with ethanol and acetate buffer at pH 6.[128] Two different approaches were tested to reduce changes in As response due to the carbon effect: addition of a supplementary methanol solution directly to the spray chamber or addition of methanol post-column through a T-piece. The latter method proved to be the best option for maintaining constant response for several arsenolipids covering a wide range of polarities. Using the optimised method it was possible to perform a screening of at least three groups of arsenolipids with different polarities in 90 min with detection limits ranging from 5 to 11 $\mu g\ As\ L^{-1}$, depending on the analysed compound. The method was applied to the screening of arsenolipids in fractions obtained from cod liver oil and capelin oil, which include As containing fatty acids, As containing hydrocarbons and another group of lower polarity and unknown character.

Two-dimensional HPLC (size exclusion followed by anion exchange) coupled to ICP-MS and ES-MS-MS has been used to investigate the *in vitro* bioaccessibility of *As species from edible seaweeds*—kombu, wakame, nori and sea lettuce.[129] The use of ammonium acetate buffer as extractant and 1 h sonication in a water bath resulted in 40–61% of the total As found in the buffered aqueous extract, of which 86–110% was present as arsenosugars (glycerol sugar, phosphate sugar and sulfonate sugar for wakame and kombu and glycerol sugar and phosphate sugar for nori). The exception was sea lettuce, for which the arsenosugar fraction (glycerol sugar, phosphate sugar) only comprised 44% of the total extracted As. After *in vitro* gastro-intestinal digestion, approximately 11–16% of the total As in the solid sample was found in the dialysates with arsenosugars comprising 93–120% and 41% of the dialysable As fraction for kombu, wakame, nori and sea lettuce, respectively. The relative As species distribution in seaweed in the buffered extracts and dialysates was found to be very similar. Arsenic speciation in edible seaweed (from the Galician coast, NW Spain) produced for human consumption has also been investigated.[130] *Chondrus crispus*, *Porphyra purpurea*, *Ulva rigida*, *Laminaria ochroleuca*, *Laminaria saccharina*, and *Undaria pinnatifida* were analysed. The study focused on arsenosugars, but As^{III} and As^{V} were also determined in aqueous extracts. Total As in the samples was determined by microwave digestion and ICP-MS. For As speciation, a water extraction suitable for arsenosugars was used, with both anionic and cationic exchange HPLC-ICP-MS detection. The total As content of the alga samples ranged from 5.8 to 56.8 mg As kg^{-1}. The mass budgets obtained in the extracts (column recovery × extraction efficiency) ranged from 38 to 92% except for *U. pinnatifida* (4%). The following compounds were detected: As^{III}, As^{V}, MMA, DMA, sulfonate sugar (SO_3-sug), phosphate sugar (PO_4-sug), AB, and glycerol sugar (Gly-sug). The highest concentrations corresponded to the arsenosugars. In a similar study, 12 commercially available edible marine algae from France, Japan and Spain and the CRM NIES no. 9 *Sargassum fulvellum* were analysed for As.[131] Total As concentrations were determined by ICP-OES after microwave digestion and ranged from 23 to 126 $\mu g\ g^{-1}$. Arsenic species in alga samples were extracted with deionised water using microwave-assisted extraction and showed extraction efficiencies from 49 to 98%, in terms of total As. The presence of 11 As species was studied using HPLC-UV-HG-AFS with both anion and cation exchange chromatography. Glycerol and phosphate sugars were found in all alga samples, at concentrations between 0.11 and 22 $\mu g\ g^{-1}$, whereas sulfonate and sulfate sugars were only detected in three of them (0.6–7.2 $\mu g\ g^{-1}$). Low concentrations of DMA (<0.9 $\mu g\ g^{-1}$) and high As^{V} concentrations (up to 77 $\mu g\ g^{-1}$) were found in most of the algae studied.

Arsenic speciation as also been studied in *other food stuffs*. Roxarsone (4-hydroxy-3-nitrophenyl)arsonic acid), used as a feed additive for poultry and swine, has again been studied.[132] HPLC-ICP-MS was used to determine As^{III}, As^{V}, MMA, DMA, (4-hydroxy-3-aminophenyl)arsonic acid, (4-hydroxy-3-acetaminophenyl)arsonic

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acid, and roxarsone in liver extracts from chickens. Roxarsone, its 3-amino and 3-acetamido metabolites, iAs, and additional unknown As species were detected. The use of feather meal product in the human food system and in other settings may result in human exposures to As. Twelve samples of feather meal product from six US states and China have been examined,[133] with HPLC-ICP-MS used to determine As species. Arsenic was detected in all samples (44–4100 μg kg^{-1}) and speciation analyses revealed that iAs dominated, representing 37–83% of total As present. Roxarsone was not detected in the samples (<20 μg As kg^{-1}). Food samples (egg, fish muscle, beef and chicken) purchased in Brazilian markets have been studied using LC-ICP-MS.[134] Samples were milled and the As species extracted by sonication for 2 min with a solution containing MeOH (10%, v/v) plus HNO_3 (2%, v/v). The LOD values for AsB, As^{III}, DMA, MMA and As^V were 1.3, 0.9, 0.6, 0.7 and 0.8 ng g^{-1}, respectively. Four CRMs, SRM1577 bovine, CE278 mussel, DOLT-3 dogfish liver tissue and DORM-3 fish protein were used for validation. Four anionic As species (As^{III}, As^V, DMA and MMA), in the presence of unretained species such as AsB have been determined in fruit juices (apple, pear, cranberry, grape (red, white and purple) juices, as well as several juice blends using IC-ICP-MS.[135] Method validation was based on repeatability, analysis of CRM materials, and recovery of fortified samples. The LOD figures were 0.35, 0.41, 0.45 and 0.70 μg kg^{-1} for As^{III}, DMA, MMA and As^V, respectively. Chromatographic recovery was good for most samples (90–107% compared to total As), though recovery for some grape juice samples was lower (67–78%).

As in previous years, there have been numerous publication reporting on the *As levels in rice* grown in various parts of the world. Carbonell-Barrachina *et al.*[136] have looked at rice-based infant foods from Spain, UK, China and USA for total and iAs using ICP-MS and HPLC-ICP-MS, respectively. The iAs contents were significantly higher in gluten-free rice than in cereal mixtures with gluten, placing infants with coeliac disease at high risk. All rice-based products displayed a high iAs content, with values being above 60% of the total As content and the remainder being DMA. Llorente-Mirandes *et al.*[137] have also looked at As in 29 samples of rice and infant cereal products using HPLC-ICP-MS. The extraction of the As species was performed using nitric acid (0.2%) and hydrogen peroxide (1%). Total As ranged from 40.1 to 323 μg As kg^{-1} and iAs species were predominant. It was found that DMA could be detected in some samples while MMA was not detected in any sample. The CRMs NMIJ 7503a, NCS ZC73008, NIST SRM 1568a were used to check the accuracy. Verification of nitric acid based extraction methods for the speciation of As^{III} and As^V in rice grain has also been reported.[138] Commercially purchased samples (121) of 12 rices were extracted using 0.28 M nitric acid at 95 °C prior to analysis by HPLC-ICP-MS with a PRP-X100 anion-exchange column (250 × 4.1 mm, 10 μm). The CRM materials NIST-1568a, NMIJ-7503a and IMEP-107 were also used. The As speciation highlighted the predominance of As^{III} in 115 samples and DMA in six samples with common minor components including As^V, MMA and two unknown As species. The high As^{III} was found in all sample types regardless of geographic origin, rice type, grain size, cultural practice and polish treatment, indicates a higher level than often to date. Extensive grinding and pressurised extraction with water have been recommended by Alava *et al.* for the effective speciation of As from rice.[139] The effectiveness of closed and open microwave digestion procedures to extract As (As^{III}, As^V, and DMA) from CRM samples of rice and 3 commercial rice matrices were evaluated together with the importance of grain particle size after grinding. Particle size was found to have a major influence on As extraction efficiency and using microwave digestion in closed vessels was better than using microwave digestion in open vessels when the particle size was larger than 0.5 mm. For powdered samples, extraction efficiencies using both methods were similar. However, less time (30 min) was needed for complete extraction using microwave digestion in closed vessels. The highest extraction efficiency for closed microwave digestion was obtained with powdered rice at 80 °C in water at a liquid/solid ratio of 10 for 30 min. Arsenic in Japanese white rice flour (20 samples) has also been determined by ICP-MS and ICP-OES after microwave-assisted digestion.[140] The total As was 0.15 ± 0.07 mg kg^{-1} (highest, 0.32 mg kg^{-1}), and the concentrations of As^{III}, As^V, and DMA, determined by HPLC-ICP-MS after a heat-assisted aqueous extraction were closely positively correlated with the total As. The daily dietary intake of iAs and some organic species has also been investigated in Japanese subjects in diet trials.[141] Diet samples were collected from 25 subjects and analysed using LC-ICP-MS and HG-AAS. A NIES CRM (NIES no. 27 Typical Japanese Diet) was also used. The median intake of iAs for the 25 subjects was 3.8 μg per day (2.0–57 μg per day), and the median intake of MMA, DMA and TMAsO were <0.18, 1.1 and <0.053 μg per day. Based on these figures, the enhanced cancer risk posed by the dietary iAs in Japan may not be negligible. Finally, As in organic brown rice syrup, used as a sweetener in organic food products as an alternative to high-fructose corn syrup has been determined by HPLC-ICP-MS.[142] Inorganic As made up 80–90% of the total As in two of the three syrups analysed, the third containing 50%. However, since the latter sample was much higher in total As, it also had the highest iAs concentration of the syrups. All syrups had detectable MMA, ranging from 3 to 4% of total As, but the major species for each syrup was DMA. The totals ranged from 80–330 ng g^{-1}. A CRM, NIST (SRM) 1568a rice flour was also used. The authors note that there are currently no U.S. regulations applicable to As in food, although organic brown rice syrup is used in many products, including toddler formula, cereal/energy bars, and high-energy foods used by endurance athletes.

Many papers have been published reporting *As levels in contaminated sites*. Many of these are based on the use of established methodologies and offer no new analytical insight, although the levels reported are often alarmingly high, for example total As concentrations up to 2271 mg kg^{-1} in plants (*P. calomelanos)* grown on an industrial site in Sepetiba Bay area of SE Brazil.[143] In some cases speciation studies are performed, such as the study by Marquez-Garcia *et al.* who used HPLC-ICP-MS to study the As speciation in soils from the Sao Domingos mine area in Portugal.[144] Two metal tolerant plant species: *Erica andevalensis*, an endemic heather, and *Erica australis*, a widely distributed species, were studied. The total content of As in soils ranged from 194 to 7924 mg kg^{-1} (dry mass), and from 1 to 24.4 mg kg^{-1} in *E. andevalensis* and from 2.7 to 11.6 mg kg^{-1} in

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E. australis. The organic forms of As were almost absent, with only DMA found in some samples of *E. andevalensis*. The plant species appeared to have different tolerance mechanisms, as *E. australis* accumulates mainly As^{III}, and *E. andevalensis* As^{V}. In a further study with a more unusual focus, As speciation in field-collected (both contaminated and uncontaminated) earthworm (*Lumbricus terrestris*) was studied.[145] Arsenic concentrations determined by ICP-MS in soils ranged from 16 to 348 mg kg^{-1}, 6.0 to 239 mg kg^{-1}, in the earthworms and 8.6 mg kg^{-1} in leaf litter sampled (all dry weight). HPLC-ICP-MS analysis revealed As^{III}, As^{V} and five organoarsenic species; AB, MMA^{V}, DMA^{V}, arsenosugar 1 (glycerol sugar), arsenosugar 2 (phosphate sugar), and TMAO in field-collected *L. terrestris.* Differences were observed in the variety of organoarsenic species present between field sites. Several organoarsenic species were observed in the leaf litter DMA^{V}, arsenosugar 2 and TMAO but not AB. Commercially sourced *L. terrestris* were also exposed to As contaminated soil in laboratory mesocosms (1.0, 98, 183, 236, 324 and 436 mg kg^{-1}) without leaf litter and were additionally analysed by XANES. Only inorganic As^{III} and As^{V} were observed. A study of surface water draining the adits at the Lucky Shot Gold Mine in Hatcher Pass Alaska[146] had elevated levels of As roughly seventy times the United States Environmental Protection Agency Drinking Water Standard of 10 μg L^{-1}, although this was rapidly diluted downstream in Craigie Creek to <2 μg L^{-1}. Despite being well oxygenated, As^{III} is the dominant As species in adit water, accounting for close to 100% of total As. The analysis was carried out using IC-ICP-MS. The determination of total As content and main inorganic and organoarsenic species found in heart tissues (auricle, mammary artery and fat) and the saphene vein of people living in As contaminated regions in Chile, has been carried out using HPLC-ICP-MS and HG-AAS.[147] The presence of high total As and high As^{III} species content in the auricle and suggested that As could be involved in the cardiovascular diseases in the region. Surface water originating from the Copahue volcano crater-lake in Argentina was analysed for total As, As^{III}, As^{V}, MMA and DMA and other trace elements (Fe, Mn, V, Cr, Ni, Zn).[148] The species were collected and preconcentrated on an SPE column (either SAX or SCX) for later analysis by HPLC-ICP-MS. A preconcentration factor of 6 gave LOD's of 0.2 μg L^{-1} for As^{III}, 0.02 μg L^{-1} for As^{V}, 0.02 μg L^{-1} for MMA and 0.02 μg L^{-1} for DMA. Validation of the technique was conducted by an intra-laboratory comparison. The highest total As levels were recorded in the el Vertedero spring (3783 μg L^{-1}) on the flank of the Copahue volcano. The speciation, however, changed from predominantly As^{III} near the crater to As^{V} further away.

Bioaccumulation, biotransformation and trophic *transfer of As in the aquatic food chain* has been reviewed by Rahman *et al.*[149] The review emphasises that to-date, most of the studies in this area have focused on marine environments and little is known about these processes in freshwater systems. However, in a very similar review published by the same authors in another journal,[150] it is also noted that eutrophication plays an important role in the production, distribution, and cycling of methylarsenic compounds in freshwaters.

Terrestrial snails are consumed by humans occasionally and they are an important food source for many creatures including fish and birds. Little is known about arsenic speciation in these gastropods, or life cycle variations. Lai *et al.*[151] have reported on the As speciation in such species from Pender Island and Vancouver Island, Canada. Methanol/water extracts (43–59% extraction efficiency) were used with HPLC-ICP-MS and HPLC-ES-MS-MS. The tetramethylarsonium ion, oxo-arsenosugars and thio-arsenosugars are the main arsenic species encountered. Arsenobetaine, commonly found in the marine environment, is minor. Live bearing snails *Viviparidae* sp. from Pender Island were maintained in aquaria and the As speciation in the unborn, newly born, and adult animals was monitored. Oxo-arsenosugars predominate in the adults, whereas thio-arsenosugars seem to predominate in juveniles, suggesting that these arsenicals are snail metabolites.

Arsenic speciation in marine samples has also been reported. The As species distribution in blue mussels (*Mytilus edulis*) obtained from an area where there is a strong As concentration gradient as a consequence of mining impacted sediments has been reported.[152] A strong positive correlation was observed between the concentration of iAs species and total As concentrations present in *M. edulis* tissues ($R^2 = 0.983$), which could result in significant toxicological consequences to the mussels and higher trophic consumers. However, concentrations of organoarsenicals, dominated by AB, remained relatively constant regardless of the increasing As concentration in *M. edulis* tissue ($R^2 = 0.307$). Bulk analysis by XANES and XAS two-dimensional mapping of wet *M. edulis* tissue revealed the presence of predominantly As–S compounds. The XAS mapping revealed that the As^{III}–S and/or As^{III} compounds were concentrated in the digestive gland. However, AB was found in small and similar concentrations in the digestive gland as well as the surrounding tissue suggesting AB may being used in all of the mussel's cells in a physiological function such as an intracellular osmolyte. The speciation studies were performed using HPLC-ICP-MS. Hamdi *et al.*[153] have reported on zebrafish (*Danio rerio*) exposed to As^{III}. The identification of several As species including MMA^{III}, MMA^{V} and DMA^{V} with characteristic tissue ratios, demonstrated that an As methylation pathway exists in zebrafish. The identification of an *S*-adenosylmethionine dependent As methyltransferase with a similar function to human and rodent orthologs in catalysing intracellular As biomethylation, indicates that zebrafish may be a valuable vertebrate model for understanding As-associated diseases in humans.

Arsenic speciation has been determined in *benthic fauna* collected from the Mid-Atlantic Ridge hydrothermal vents.[154] The shrimp species, *Rimicaris exoculata*, the vent chimney-dwelling mussel, *Bathymodiolus azoricus*, *Branchipolynoe seepensis*, a commensal worm of *B. azoricus* and the gastropod *Peltospira smaragdina* showed variations in As concentration and in stable isotope (C^{13} and N^{15}) signature between species, suggesting different sources of As uptake. Arsenic speciation showed AB to be the dominant species in *R. exoculata*, whereas in *B. azoricus* and *B. seepensis* arsenosugars were most abundant, although AB, DMA and iAs were also observed, along with

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several unidentified species. The formation of arsenosugars in pelagic environments is typically attributed to marine algae, and the pathway to AB is still unknown. The occurrence of arsenosugars and AB in these deep sea organisms, where primary production is chemolithoautotrophic and stable isotope analyses indicate food sources are of vent origin, suggests that organic As compounds can occur in a foodweb without algae or other photosynthetic life. The As content of sediments, detritus, suspended particles and organisms have been investigated from different trophic levels in an open seagrass ecosystem.[155] Total As concentrations and As species were determined by HPLC-ICP-MS and found to be organism-specific and determined by a variety of factors including exposure, diet and the organism physiology. Sediments, seagrass blades and detritus contained mostly iAs (50 to 90 %) and arsenoribosides (10 to 26 %), with some MMA (9.4 to 14.6 %) and DMA (7.9 to 9.7 %) in seagrass blades and detritus. Macroalgae contained mostly arsenoribosides (40 to 100%). Epibiota and other animals contained predominately AB (63 to 100 %) and varying amounts of DMA (0 to 26%), MMA (0 to 14.6%), iAs (0 to 2%), trimethylarsenic oxide (0 to 6.6%), AC (0 to 12%) and tetramethylarsonium ion (0 to 4.5%).

Arsenic speciation in biomedical sciences, with emphasis on the specimens commonly encountered in biomedical laboratories has been reviewed by Hsu *et al.*[156] Ingestion of water containing inorganic iAs has been linked to a variety of adverse health effects, including cancer, hypertension and diabetes. Current evidence suggests that the toxic methylated trivalent metabolites of iAs, MMA^{III} and DMA^{III} play a key role in the etiology of these diseases. Both MMA^{III} and DMA^{III} have been detected in urine of subjects exposed to iAs. However, the rapid oxidation of DMA^{III} and, to a lesser extent, MMA^{III} in oxygen-rich environments leads to difficulties in the analysis of these metabolites in samples of urine collected in population studies. Currier *et al.*[157] have used HG-CT-AAS to examine the presence and stability of these trivalent metabolites in the liver of mice and in UROtsa/F35 cells exposed to iAs following storage of up to 22 days and various temperatures. Tri- and pentavalent metabolites of iAs were analysed directly (without chemical extraction or digestion). The results suggest that samples of cells and tissues represent suitable material for the quantitative, oxidation state-specific analysis of As in laboratory and population studies examining the metabolism or toxic effects of this metalloid. Studies have revealed that microorganisms from the gut environment are important contributors to As speciation changes. The biotransformation of As using a Simulator of the Human Intestinal Microbial Ecosystem (SHIME) has been reported by Alava *et al.*[158] who used HPLC-ICP-MS, employing a Hamilton PRP-X100 anion exchange column, to separate As^{III}, As^{V}, DMA^{V}, MMA^{V} and MMMTA (monomethylmonothioarsenate). Attempts to optimise the same method for also separating MMA^{III} and DMA^{III} did not succeed. These compounds could be successfully separated using a RP C_{18} column. The combination of the two methods allowed successful quantification of As species in suspensions sampled *in vitro* from the SHIME reactor or *in vivo* from the human colon and faeces.

Possible key intermediates in *As biochemistry* have been reported by de Bettencourt *et al.* using LC-ES-MS and high resolution MS.[159] The study focused on the thiol protein As bond and on its interaction with reactive metabolites. Various As complexes were identified and the results obtained suggest that cell vacuoles might play a major role in As metabolism, and that the dominance of oxo-As sugars, in algae extracts, may be supported by a mechanism of synthesis independent of the precursor dimethylarsonio-adenosine glutathione DMAAG (*m*/*z* 661). A HG-CT-AAS method was used to speciate As in an injectable drug, *N*-methylglucamine antimonite.[160] The method employed generation of substituted hydrides and selective HG, which makes possible an analysis of As^{III} and As^{V} and their mono-, di-, and trimethyl substituted species. Interference of the Sb matrix was eliminated using the T-valve to prevent supply of the stibine interferent to the atomiser. The LOD in measured sample solutions were 70 ng L^{-1} for As^{V}, 42 ng L^{-1} for MMA and DMA and 30 ng L^{-1} for all the other determined species. Concentrations of all methylated species in all samples were below their LOD. A significant but different amount of iAs was found in different batches of the drug: between 0.9 and 2.3 mg L^{-1} with 7% to 10% of the content present as the trivalent form. The accuracy was assessed by the comparison of the determined iAs content with the total As content determined by ICP-MS. The HG-CT-AAS procedure showed good performance with minimum sample pre-treatment at low investment and running costs. A study in Taiwan evaluated the As methylation profile of adolescents and explored the influence of body mass index (BMI) on the As methylation profile of adolescents in an area of Taiwan with no-obvious as exposure.[161] This study evaluated 202 normal weight students. Concentrations of urinary As species, including iAs, MMA and DMA were determined by a HPLC-HG-AAS. Urinary total As was significantly decreased with increasing BMI, indicating that obese children may retain higher levels of As in the body. Participants with obesity accompanied by high insulin levels had higher iAs, significantly higher MMA percentage and significantly lower DMA percentage than those with obesity and low insulin levels. It seems children with obesity and high insulin levels had lower As methylation capacity than those with obesity and low insulin. This is the first study to demonstrate that total urinary As is negatively associated with the BMI in adolescents.

The *speciation of As in human urine* continues to attract attention since high iAs concentrations in drinking water has been related to detrimental health effects, including cancers and possibly cardiovascular disease, in many epidemiological studies. The strong heart study, a large epidemiological study of cardiovascular disease in American Indian communities, collected urine samples and performed medical examinations on 4549 participants over a 10 years period beginning in 1989.[162] Anion-exchange HPLC-ICP-MS was used to determine concentrations of As species (MMA, DMA and As^{V}) in 5095 urine samples. A portion of the urine sample was oxidised, by addition of H_2O_2, to provide additional information on the presence of As^{III} species and thio-arsenicals, and by difference, of AB and other non-retained cations. Total concentrations for As, Cd, Mo, Pb, Sb, Se, U, W, and Zn were also determined by ICP-MS. The dataset will be used to evaluate the relationships between the concentrations of urinary As species and selected metals with

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various cardiometabolic health endpoints. Internal standardisation based on a species-unspecific IDA technique has been proposed to overcome the matrix effects and signal drift originated in the speciation of As in urine by HPLC-ICP-MS.[163] To this end, Ge^{72} was selected as a pseudo-isotope of As. The resulting mass flow chromatogram of the element allows the calculation of the corrected overall species concentrations without requiring any methodological calibration, providing high-throughput sample processing. Validation was carried out by analysing a blank human urine fortified at three concentration levels and an unspiked human urine sample containing different species of As. In all cases, recoveries ranging from 90 to 115% and RSD below 10% were attained. The proposed method provided results in excellent agreement with those obtained using standard additions and internal standard calibration, allowing a fast way to assess human exposure to As species. Speciated urinary As has also been used as a biomarker of dietary exposure to iAs in 153 residents living in high-As areas in Latium, Italy.[164] The study using HPLC-ICP-MS showed, iAs and related metabolites constituted 75% of total urinary As (2 to 72 $\mu g\ L^{-1}$). AB and other organoarsenic compounds were detected at low concentrations in all urine samples except those with high seafood consumption. The study also detected dimethylthioarsinic acid (DATA) in 33% of the samples at levels up to 6 $\mu g\ L^{-1}$. The study also found that part of the certified DMA content of human urine reference material SRM 2669 was present as DATA. Four unknown arsenicals were also detected as minor species in a small number of samples.

The *speciation of As trioxide metabolites in peripheral blood and bone marrow* from an acute promyelocytic leukemia patient has been conducted.[165] The As speciation in plasma from bone marrow was compared with those of peripheral blood plasma from a relapsed acute promyelocytic leukemia patient. The analyses of total As concentrations and speciation were performed by ICP-MS, and HPLC-ICP-MS respectively. The results suggested that As speciation analysis of peripheral blood plasma could be predictive for bone marrow speciation, and showed relatively higher efficiency of drug metabolism in the patient. The results may provide further evidence of the significance of clinical application of As trioxide, but also a new insight into host defence mechanisms in acute promyelocytic leukemia patients undergoing AS trioxide treatment. In particular, high molecular weight protein-bound-As complexes might protect bone marrow from the attack of free As species.

5.3 Chromium

The *speciation of Cr in environmental samples*[166,167] and the preconcentration techniques used for the determination of Cr species by atomic spectroscopy[168] have both recently been reviewed. Chromium speciation has continued to attract widespread attention in this review period and applications in a range of matrices have been reported.

The determination of *Cr^{VI} in corrosion protection coatings* by ID-ICP-MS has been reported by Novotnik *et al.*[169] The investigation was performed on Cu or Zn electroplated steel surfaces homogeneously treated by Cr conversion or hard chrome coatings. An alkaline solution (pH 12) was used for ultrasonic extraction of Cr^{VI}. Speciation analysis was performed by anion-exchange HPLC-ICP-MS. Species interconversions during the analytical procedure were followed using enriched isotopic solutions of Cr-50(VI) and Cr-53(III). To prevent Cr^{III} oxidation, Tris, EDTA or $MgCl_2$ was added and extractions were performed over different time periods. Under optimal conditions that prevented any species interconversion (30 min ultrasonic extraction at 70 °C using 2% NaOH + 3% Na_2CO_3 + $MgCl_2$ as an extraction agent), six consecutive extractions were necessary to quantitatively extract Cr^{VI} from the protective layers. Concentrations of Cr^{VI} in the samples investigated ranged from 2 to 7 ng per mm^2. The accuracy of the HPLC-ICP-MS determinations was checked by analysis of CRM 545, Cr^{VI} in welding dust, using both external calibration and ssID-ICP-MS. Good agreement was obtained between the determined and the certified values (±0.7% for external calibration and ±0.2% for ssID-ICP-MS). The high sensitivity of the procedure developed (LOQ 0.0107 ng Cr^{VI} per mm^2 on a surface of 250 mm^2) and the possibility to use external calibration for quantification of separated Cr^{VI} instead of ssID-ICP-MS allows its application to be extended to the routine laboratory.

The *use of SPE continues to be popular for Cr speciation*. A non-chromatographic system has been developed for the simultaneous determination of Cr^{III} and Cr^{VI} by SPE and ICP-MS.[170] The system is based on the use of three minicolumns packed, respectively, with chelating and anionic ion exchange resins, which were placed in the injection valve of a simple flow manifold. Detection limits (2 min sample loading time) were reported of 0.009 $\mu g\ L^{-1}$ for Cr^{VI} and 0.03 $\mu g\ L^{-1}$ for Cr^{III}. The relative standard deviations ($n = 10$) for 0.3 $\mu g\ L^{-1}$ of Cr^{VI} and Cr^{III} were 3.2% and 2.6%, respectively. The accuracy of the proposed method was checked with certified reference materials and the method was applied to the determination of Cr species in different seawater samples. Dadfarnia *et al.* have reported on the use of SPE on immobilised ferron.[171] For the determination of the total concentration of Cr in solution, Cr^{VI} was efficiently reduced to Cr^{III} by addition of hydroxylamine and Cr^{III} was preconcentrated on a column of immobilised ferron on alumina. The adsorbed analyte was then eluted with 5 mL of hydrochloric acid and was determined by FAAS. The speciation of Cr was affected by first passing the solution through an acidic alumina column which retained Cr^{VI} and then Cr^{III} was preconcentrated by immobilised ferron column and determined by FAAS. The concentration of Cr^{VI} was determined from the difference of concentration of total Cr and Cr^{III}. The effect of pH, concentration of eluent, flow rate of sample and eluent solution, and other ions on the sorption of Cr^{III} by the immobilised ferron column was investigated. Under the optimised conditions the calibration curve was linear over the range of 2–400 $\mu g\ L^{-1}$ for a 1000 mL preconcentration volume. An LOD of 0.32 $\mu g\ L^{-1}$ and preconcentration factor of 400 was achieved with an RSD of 1.9% (at 10 $\mu g\ L^{-1}$; $n = 7$). The method was successfully applied to the determination of Cr species in water samples and total Cr in standard alloys. The SPE of Cr^{VI} from aqueous samples using Aliquat336 immobilised on a thin film of multiwall carbon nanotubes has been reported.[172] The Cr was

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determined by XRF. The enrichment factor (1000) was calculated after considering that the thin film obtained from the 10 mL solution of 1 mg L^{-1} of Cr^{VI} had a real thickness of 0.04 mm and a final diameter of 16.7 mm, so that the volume deposited on the pellet was 0.0088 cm^3. *N*,*N'*-bis-(alpha-methylsalicylidene)-2,2-dimethyl-1,3-propanediimine (SBTD) modified silica gel has been used as a sorbent for SPE of Cr^{III} ions from aqueous solution.[173] This sorbent showed a high sorption affinity for Cr^{III} while recovery of Cr^{V} was very low. The analyte ion retained on the column was eluted with 1 mol L^{-1} HNO_3. The Cr ion in the eluent was determined by ETAAS. The effects of different parameters such as pH, eluent type and volume, Schiff's base concentration, sample and eluent flow rate, interfering ions and adsorbent amount were also investigated.

The *determination of low levels of Cr^{III} and Cr^{VI} by dispersive LLE* using *in situ* formation of an ionic liquid followed by ETAAS has been reported.[174] The ionic liquid, 1-octyl-3-methylimidazolium bis(trifluoromethanesulfonyl) imide ([C_8MIm]-[NTf_2]), was formed *in situ* by mixing 1-octyl-3-methylimidazolium chloride ([C_8MIm]Cl) and lithium bis(trifluoromethanesulfonyl) imide ([NTf_2]Li) aqueous solutions, and was used to extract complexes originating from Cr^{III} and Cr^{VI} with APDC. No organic solvents were required, the dispersion being due to the very fine droplets of ([C_8MIm][NTf_2]) originated in the aqueous solution. After centrifugation, the concentration of Cr in the sedimented phase was determined by ETAAS. The total content of Cr was measured using 0.01 mol L^{-1} APDC at pH 1. In the presence of 0.001 mol L^{-1} ethylenediaminetetraacetate, Cr^{III} is not extracted, and the analytical signal corresponds to Cr^{VI}. The level of Cr^{III} was obtained by difference. An enrichment factor of 300 and an LOD of 2 ng L^{-1} was achieved. The relative standard deviation for 10 replicates at the 0.05 μg L^{-1} level was close to 8%, and the recoveries of spiked samples were in the 95–104% range. The method was applied to the analysis of water samples as well as lixiviates obtained from toys made of plastic materials. The preconcentration and speciation of sub ng L^{-1} levels of Cr species in aqueous solutions with high salt contents has been described.[175] A temperature-controlled microextraction of Cr species using the 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF_6]) ionic liquid as an extractant was followed by determination employing ETAAS. The extraction of Cr species from aqueous solution into the fine droplets of [HMIM][PF_6] was performed with APDC as the chelating agent. Under optimum conditions, the calibration graphs were linear over the concentration ranges from 50 to 200 ng L^{-1} for Cr^{III} and from 25 to 150 ng L^{-1} for Cr^{V}. The LODs of the developed method were 5.40 ng L^{-1} and 2.45 ng L^{-1} for Cr^{III} and Cr^{V} ions, respectively. The enrichment factor for Cr species was 42. The RSD for six replicate determinations of 100 ng L^{-1} of either Cr^{V} or Cr^{III} were 4.24% and 3.05%, respectively. The method was successfully applied to the speciation and determination of Cr species in water and urine samples. The salt-assisted LLME of cationic complexes of Cr^{VI} ion has also been achieved using the hydrophilic ionic liquid 1-butyl-3-methylimidazolium tetrafluoraborate and potassium hydrogen phosphate.[176] The Cr^{VI} was complexed with 1,5-diphenylcarbazide (DPC) in sulfuric acid medium. It was applied to the extraction of Cr^{VI} in the form of the Cr^{VI}–DPC complex prior to its determination by FAAS. The Cr^{III} ion could also be determined by this procedure after oxidation to Cr^{VI}. Extraction efficiency was mainly affected by the amount of water-soluble ionic liquid, the type and quantity of inorganic salts, pH and the concentration of DPC. Calibration plots were linear in the range from 3 to 150 μg L^{-1} of Cr^{VI} and the LOD reported was 1.25 μg L^{-1}. The method was successfully applied to the speciation and determination of trace levels of Cr^{III} and Cr^{VI} in environmental water samples containing high levels of dissolved salts or food grade salts.

A procedure has been developed for the *speciation of Cr by carrier element co-precipitation* and DLLME coupled with microsample injection system-FAAS.[177] Thus, APDC, carbon tetrachloride and ethanol were used as chelating agent, extraction solvent and disperser solvent, respectively for the determination of Cr^{V}. For total Cr, Cr^{III} was oxidised by $Ce(SO_4)_2$ in acidic media (0.07 mol L^{-1} H_2SO_4) and the resulting solution was co-precipitated with APDC. The concentration of Cr^{III} was estimated by determining the difference between the concentration of total Cr and that of Cr^{V} The maximum recovery of Cr^{V} was obtained with DLLME at optimal conditions of pH 3.0, 0.25% APDC, 100 μL CCl_4, 1.00 mL of CH_3CH_2OH and 0.01 mg L^{-1} Cr^{V}, whereas, the optimal conditions by carrier element co-precipitation were 40 mL initial volume of water samples, 0.25% APDC, 0.02% $Ce(SO_4)_2$ and 0.10 mg L^{-1} Cr^{V}. The LOD and enrichment factor of DLLME and carrier element co-precipitation were 0.037 and 2.13 μg L^{-1} and 400 and 100, respectively with 40 mL initial volumes. The RSD values ($n = 6$) were <4%. The proposed method was successfully applied to the Cr speciation at ultra-trace levels in natural drinking water and industrial effluent waste water. Activated carbon, chemically modified with Tris(hydroxymethyl) aminomethane, has been used as the absorbent for Cr species in natural waters prior to determination by ICP-AES.[178] The maximum static adsorption capacity of Cr^{VI} was found to be 43.3 mg g^{-1} at pH 1 and a 1 hour contact time. The adsorption data of Cr^{VI} were modelled using both Langmuir and Freundlich classical adsorption isotherms to reveal that the adsorption of Cr^{VI} followed a pseudo second-order kinetic model. The determination of Cr^{III} and Cr^{VI} in natural waters based on co-precipitation of Cr^{III} with Pr^{III} hydroxide and determination by FAAS has also been reported.[179] The Cr^{III} was quantitatively (>95%) recovered in the pH range 10.0–12.0 on Pr^{III} hydroxide, although the recoveries of Cr^{VI} were below 10%. The method was applied to the determination of the total Cr after reduction of Cr^{VI} to Cr^{III} by using hydroxylamine hydrochloride. The concentration of Cr^{VI} is calculated by difference of total Cr and Cr^{III} levels. Modified nano polyacrylonitrile fibres have been prepared to preconcentrate Cr^{III} from natural waters.[180] The fibres were prepared by adding acrylic fibres to methanolamine (MMA) and detection, following preconcentration by a factor of 100, was by FAAS. Cr^{III} at levels of 60 pg mL^{-1} were found in local tap water.

A *modified form of chitosan* was synthesised using 2-hydroxyethyltrimethyl ammonium chloride for the adsorption of Cr^{VI} in aqueous solutions.[181] The material could preconcentrate Cr^{VI} at pH 4.0; the adsorption equilibrium time was 80 min; the

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maximum adsorption capacity was 205 mg g^{-1}. The method was used with FAAS to determine Cr^{VI} and Cr^{III} in environmental water samples. The LOD reported was 20 ng L^{-1} with an RSD of 1.2% and recovery 99% to 105%. The adsorption properties of cross-linked magnetic chitosan anthranilic acid glutaraldehyde Schiffs base resin toward both As^{V} and Cr^{VI} has also been evaluated.[182] Various factors affecting the uptake behaviour such as pH, temperature, contact time, initial concentration of metal ions, effect of other ions and desorption were studied. The equilibrium was achieved after about 110 min and 120 min for As^{V} and Cr^{VI}, respectively at pH = 2. The ions were determined by ICP-OES although SEM, FTIR, wide angle XRD and TGA analysis were used to investigate cross-linking on the resin.

A number of *environmental studies* have focused on Cr speciation. A study of the emissions from steel mills in Germany has shown enhanced concentrations of Cr^{VI} in ambient air.[183] Sampling was carried out using impingers filled with a slightly alkaline phosphate/aluminium sulphate buffer which suppressed redox reactions of Cr. Diphenylcarbazide was added to the solution in order to form a complex with Cr^{VI} which was extracted and analysed by ICP-MS. The diphenylcarbazide complex also ensured an effective separation of Cr^{VI} from Cr in other oxidation states. The percentage of Cr^{VI} related to total Cr was, on average, 3.2% during the study. In another report, *Gynura pseudochina (L) DC.*, a Cr tolerant plant, has been suggested as a potential candidate for phytoremediation.[184] To understand the tolerance mechanism, the study investigated the speciation and distribution of Cr accumulated in *G. pseudochina (L) DC.* using AAS, XAFS, μXANES, and μXRF imaging. The plants were separately treated with $K_2Cr_2O_7$ and $Cr_2(SO_4)_3$ in a hydroponic system. The study showed that Cr^{III} and Cr^{VI} were taken up into the vascular system and transported from the roots to the leaves. $Chromium^{III}$ was distributed *via* the symplast system to the ground tissue and accumulated mainly in the stem cortex whilst Cr^{VI} was transported to the xylem *via* the apoplast system. The adsorption of Cr^{VI} and its reduction to Cr^{V} and Cr^{III} occurred on oxygen ligands in the lignocellulosic structure of the xylem and vein.

Chromium-tanned leather footwear, which releases in excess of 3 mg kg^{-1} Cr^{VI}, into a phosphate buffer at pH 7.5–8.0 when extracted according to ISO 17075 methodology, may pose a risk of sensitising and eliciting allergic dermatitis. This has been the subject of a risk assessment by Thyssen *et al.*[185] who studied 60 pairs of leather shoes, sandals and boots (20 children's, 20 men's, and 20 women's) using XRF. Chromium was extracted according to ISO 17075 and identified in 95% of leather footwear products. No relationship *with Cr content* was found with footwear category, although there was a tendency for there to be a higher Cr (up to 62 mg kg^{-1} Cr^{IV}) content in footwear with high prices. The study indicated that most leather footwear contained Cr, often as Cr^{VI}, thus posing a risk of sensitisation. Chromium-contaminated effluents from the leather industry have been studied by several groups. Sediment pore waters were investigated in a river adjacent to the property of a large former tannery, into which Cr-contaminated effluent was discharged over a 55-year period in Canada.[186] Dialysis cells were used to generate depth profiles of Cr concentration in sediment pore water. Samples were analysed for total Cr using ICP-MS and for Cr species using HPLC-ICP-MS. The results showed an absence of Cr^{VI} in all samples. Furthermore, incomplete recovery of Cr^{VI} added to the samples collected at the locations with highest sediment Cr concentrations indicated strong reducing conditions at those locations. Pereira *et al.*[187] used FI-AAS to study tannery discharges into rivers. Different preconcentration times and complexing agents were adopted for each species: 360 s and xylenol orange for Cr^{III}, and 1,5-diphenyl carbazide and 600 s for Cr^{VI}. Methanol was used as the eluent. The concentration of Cr^{III} in the water samples collected ranged from 5.2–105.2 μg L^{-1}, although Cr^{VI} was always below the LOD (0.3 mg L^{-1}). Chromium accumulation observed in the sediment ranged from 873–1690 mg kg^{-1} and from 449–9320 mg kg^{-1} in particulate material.

5.4 Gadolinium

An interesting new approach[188] to simulate oxidative phase I metabolism, such as is catalysed by the cytochrome P450 enzymes and based on online electrochemistry (EC) coupled to ES-MS, has been applied to the *identification and quantification of the potential metabolites of Gd-based contrast agents*. The oxidative pathways studied focused on the five most frequently used contrast agents for magnetic resonance imaging (MRI): gadopentetate (Gd-DTPA); gadodiamide (GD-DTPA-BMA); gadobenate (Gd-BOPTA); gadoterate (Gd-DOTA); and gadobutrol (Gd-BT-DO3A). Mass voltammograms generated with online EC-ES-MS gave a first overview of oxidation products. Two potential metabolites could be detected, with the major metabolite originating from an *N*-dealkylation (M1). The four other Gd complexes showed similar reactions in the EC-ES-MS set-up. A wide range of separation and detection techniques were applied in further experiments. The Gd–DTPA complex and its *N*-dealkylation product were successfully separated by CE coupled to ES-MS or ICP-MS and, under the conditions used, 8.8% of Gd-DTPA was oxidised. Experiments with CE indicated that the second oxidation product, M2, detected in the mass voltammogram, was unstable and decomposed to M1 and further online experiments with HPLC-ES-MS confirmed the decomposition of M2. Time-resolved measurements showed a decrease of M2 and a simultaneous increase in M1 within only a few minutes, confirming the conclusion that M2 degrades to M1 while EC-HPLC-ICP-MS measurements provided quantitative evidence of this as well.

5.5 Gold

Despite the antirheumatic properties of *the gold-containing drug Auranofin* (2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*-(triethyl-phosphine) gold(I)) being reported in the early 1980s, it is still currently used in the treatment of rheumatoid arthritis. Albert *et al.*[189] have used HPLC-ICP-MS and an Orbitrap ES-MS to study the *in vitro* interaction of the drug with two thiol-containing compounds, glutathione and HSA. In aqueous solutions (pH 7.4 buffer) of Auranofin, three gold species could be identified of which the monomeric form [AtgS–Au–PEt_3] was the most abundant. The Auranofin was then reacted *in vitro* at pH

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7.4 with both glutathione and the related glutathione disulfide analogue. Upwards of 11 compounds were identified by HPLC-ES-MS and 17 products containing ^{197}Au by HPLC-ICP-MS. However the main Au-containing peak comprised two of the adducts which co-eluted from the column. Unfortunately no further HPLC work was reported so it is unclear which of the two adducts was formed in solution or whether this occurred during the separation. The drug was also incubated with HSA again at pH 7.4 and studied by the complementary MS methods. However, the resolving power of the ES instrument was not able to fully separate the ions sufficiently for deconvolution of the pattern to produce a clear understanding of the drug–protein reaction. The authors did report a single Au containing adduct identified as the triethylphosphine gold moiety bound to a Cys-34 residue of mercaptoalbumin. Whilst the thiol ligand of Auranofin was displaced, the remaining triethylphosphine gold structure was covalently bound to either glutathione or HSA. Moreover, oxidation of the phosphine ligand by disulfides occurred in both reaction mixtures with producing phosphine oxide. Reactions of Auranofin with the thiols were quantitatively traced over several days by ICP-MS. Based on the data obtained, a potential reaction pathway of Auranofin with the two thiols was proposed. However the paper does not describe patient data so it is difficult to put these simple *in vitro* studies into context. The initial aqueous experiment was conducted at pH 7.4, and showed that the mono adduct was the main activated drug, However this proposition may be flawed for the *in vivo* situation, because this is an oral drug and presumably therefore comes into contact with stomach acids at a much lower pH.

5.6 Halogens

Interest in the speciation of chemical species containing halogens (Br, Cl or I) has again been apparent this year. Meermann *et al.*[190] *speciated the Br containing anti-tuberculosis drug TMC207* in faeces collected within a clinical study. The drug is a diarylquinoline, containing a single atom of Br, with a new mode of action against mycobacteria targeting the ATP synthase. It is metabolised to an active derivative, *N*-desmethyl-TMC207, and both compounds are eliminated with long half-lives reflecting slow release from tissues. Quantification of the Br species was accomplished using HPLC-ICP-MS in combination with on-line IDMS using species unspecific spiking, while structural elucidation was obtained using HPLC-ES-MS/MS. The ICP-MS method showed a good intra- and inter-day reproducibility (RSD 3.5%, $n = 9$) and the LOD of 1.5 mg TMC207 L^{-1} was of the same order of magnitude as that for HPLC with radioactivity detection, whereas the dynamic range covered more than two orders of magnitude. Column recovery between 90.6 and 99.5% demonstrated quantitative recoveries for the Br-containing compounds. An added advantage was that the method eliminated the need to use a ^{14}C radiolabel in the human *in vivo* trial, which would be problematic for drugs with a long residence time in the body. In a related piece of work[191] the same Br-containing drug, but in this case containing an isotopically enriched analogue (^{81}Br 99.62%), was administered to rats. Faecal samples were analysed using HPLC-ICP/MS and reverse IDMS *via* species unspecific spiking. The rationale in this approach was that reverse ID allows a distinction between endogenous and exogenous compounds containing the same target element to be made on the basis of the measured isotope ratio. The advantages of this seem minor compared to the costs involved, particularly as the same degree of chromatographic specificity would be required with or without this approach. It is also unclear why there would be any endogenous contribution to a drug of this type, but also loss of the enriched isotope during metabolism would effectively make the drug invisible to the ICP-MS detection system. This approach may have application in some specific drug metabolism and pharmacokinetic studies, but these drawbacks would need to be considered before use. In a final paper on the subject[192] the group mapped the distribution of the drug and its metabolites across thin sections of rat after administration of the Br-containing drug. The sacrificed animal was frozen and embedded in carboxymethyl cellulose (CMC), after which thin sections were taken using a microtome. For quantification purposes gelatine was doped with Br and hardened into a film on a glass support for use as "matrix-matched" Br standards. The LOD was 0.1 μg g^{-1}, sufficiently low to allow visualisation of Br in the main organ of drug action the lung.

A comprehensive review of *I species in the environment*, biology and nutrition has been published.[193] The paper documented investigations related to total I but also speciation using non-chromatographic methods as well as HPLC, GC and CE coupled to electrochemical, UV spectrophotometric and MS detection. The review highlighted the range of I containing species that have been studied including: iodide; iodate; periodate; 3-iodo-tyrosine (MIT); 3,5-diiodo-tyrosine (DIT); the thyroid hormones T3 and T4; methyl-iodide; as well as more esoteric species such as: perfluorinated iodine alkanes; a range of iodinated acrylates, polyphenols, acetone and unspecified biomolecules of varying size. Clearly the speciation of I is an active area of research particularly in environmental, nutritional and clinical studies. The speciation of I in the marine environment is by far the major focus for work in this area and methods for the analysis of seawater and seaweed, including edible varieties, are predominant in the literature. Work on the speciation of I in the marine environment has looked at the isotopic fractionation[194] of ^{129}I and ^{127}I between iodide and iodate in the surface waters of the Baltic Sea. Extensive separation methods were used, followed by determination of the ^{129}I isotope by AMS and ^{127}I by conventional ICP-MS. The ratios of $^{129}I^- : ^{129}IO_3^-$ and $^{127}I^- : ^{127}IO_3^-$ were found to significantly increased from the southern to the central Baltic Sea and I^- (both isotopes) was the predominant inorganic I species. A hyphenated HPLC-ICP-MS approach was used by Han *et al.*[195] for the speciation of I^- and IO_3^-, but also two iodinated amino acids, MIT and DIT, in samples of marine origin. The ion-pair reversed phase system deployed used 2 mM tetraethylammonium hydroxide, 10 mM L-phenylalanine and 1% methanol (pH 8.9) as the eluent. The separation was achieved within 5 min due to the column temperature (25 °C) and the flow rate used 1.5 mL min^{-1}. The LOD values for IO_3^-, I^-, MIT and DIT were 0.061, 0.052, 0.079 and 0.24 μg L^{-1} respectively, and the

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repeatabilities at concentrations of 20 μL^{-1} for iodide and iodate, 40 μL^{-1} for MIT, 100 μL^{-1} for DIT, were all lower than 4%. Recoveries of the inorganic species, MIT and DIT in seaweed and seawater samples were reported between 90 and 110%. The method was successfully applied to the speciation analysis of I in seaweed and seawater samples. The bioaccessibility of I and Br in edible seaweeds[196] was assessed *in vitro* using the dialysability of the different species. Anion exchange chromatography coupled to ICP-MS, using 175 mM ammonium nitrate plus 15% (v/v) methanol, pH 3.8, as a mobile phase, was used to separate IO_3^-, I^-, MIT and DIT, along with Br^- and BrO_3^-. Low dialysability ratios (within the 2–18% range) were found for the I species; whereas, moderate dialysability percentages (from 9.0 to 40%) were obtained for the Br species. The major species found in the dialysates from seaweed were I^- and Br^-, although MIT and BrO_3^- were also found in most of the seaweed samples analysed. However, DIT was only found in dialysates from Wakame, Kombu, and NIES 09 (Sargasso) CRM; whereas, IO_3^- (iodate) was not found in any dialysate. The dialysability of I was found to be dependent on the protein content (negative correlation), and on the carbohydrate and dietary fibre levels (positive correlation). However, Br dialysability was only dependent on the protein amount in seaweed (negative correlation).

The *volatile Cl containing compounds* chlorobenzene, 1,2-dichlorobenzene and 1,4-dichlorobenzene along with toluene and their deuterated analogues, have been measured[197] using GC-AES. The novel finger sized emission detector used a helium radio-frequency plasma and a small CCD spectrometer with optical resolution of 0.035 nm. Due to the instrumental optical resolution of 0.035 nm the atomic emission lines of hydrogen and deuterium at 656.28 nm and 656.10 nm were not resolved. However, the compounds were resolved chromatographically on the capillary GC column used. The flow rate of the make-up (helium) gas, applied power and frequency were optimised at 5 mL min^{-1}, 20 W and 220 kHz, respectively. The LODs (3σ) for hydrogen in toluene and deuterium in toluene-d_8 were estimated to be 28 pg and 42 pg, respectively. To test the system aromatic pollutants in tap water were extracted by head-space SPME and successfully determined at the $\mu g\ L^{-1}$ level using deuterium-substituted analogues as internal standards.

5.7 Mercury

A review concerning the use of species-specific and species-unspecific IDA, both for the quantification of mercury species in seafoods has been published.[198] The paper covers the theory and practical implementation of IDA steps required, including in particular species stability and spike equilibration with the natural sample, to provide accurate results. The authors conclude, as have many other workers, that the use of IDA allows the identification of the critical steps in an analytical method and point out that the extent of inter-species conversions can be matrix dependent. The speciation of Hg in environmental and biological samples with atomic spectrometric detection has been the subject of a review. The authors also emphasised that the key step in achieving successful speciation of Hg lay in sample preparation and analyte extraction.[199] The review covered topics including GC, HPLC, CE and also non-chromatographic methods for the separation of Hg species published since 2008. It was evident that ICP-MS and AFS were the most commonly used detectors in this time period. It was also concluded that future advances in this field should focus on miniaturised, automated instrumentation leading to reagent-free analysis.

The application of *ss-ID-GC-ICP-MS for the determination of total Hg and MeHg in seafood* samples representative of those consumed in France has been described.[200] Three different CRMs (TORT-2, DOLT-4 and BCR-464) were each subjected to three different spiking protocols, termed double IDA, double SS-IDA and isotope pattern deconvolution. Three different extraction methods were used: (a) heating at 85 °C for two hours, (b) MAE at 60 W for three minutes and (c) standing at room temperature for 24 hours, with TMAH employed as the extractant in each case. The spike isotopes used were ^{199}Hg for inorganic Hg and ^{201}Hg for MeHg. Mercury species were simultaneously derivatised and extracted *in situ*, with 1% sodium tetrapropyl borate ($NaPr_4B$) in a sodium acetate/acetic acid buffer at pH 4 into isooctane, prior to GC separation and isotope ratio measurements by ICP-MS. A MXT-1 column (length 30 m, i.d. 0.53 mm, film thickness 1 μm) was used for the separation of mercury species prior to the isotope ratio measurements with Tl added, presumably post column in the liquid phase, to monitor mass bias. The total Hg and MeHg concentrations found were generally in good agreement with the certified values of the CRMs for all three extraction protocols. Heating the samples at 85 °C for two hours was found to be the optimal extraction technique, with respect to the precision obtained and minimised mercury species transformations, and was used for the rest of the study. The method was further validated by participation in external proficiency testing schemes, FAPAS 07136 and IMEP-109, with Z scores of 0.6 and 0.3 for total Hg and 0.8 and −0.6 for MeHg being obtained respectively for each PT scheme. Finally, MeHg and total Hg was determined in seven different composite fish and shellfish species. The fish/shellfish samples were prepared as for human consumption, to account for species transformations during cooking, before the extraction procedure was performed. The concentrations found ranged from 5 $\mu g\ kg^{-1}$ MeHg as Hg for Great Scallops up to 249 $\mu g\ kg^{-1}$ MeHg as Hg for Tuna fish. The estimated LOQs were 1.2 μg Hg kg^{-1} for MeHg and 1.4 μg Hg kg^{-1} for total Hg. It was reported that significant mercury species transformation was only observed for one oyster sample resulting in 18% demethylation of inorganic Hg. In a separate paper,[201] double spiking IDA was also described in the investigation of UAE of Hg species in fish samples. Four different extraction solutions, (5 mol L^{-1} HCl, 60 mmol L^{-1} HCL in saturated NaCl, 3.0 mol L^{-1} KOH in MeOH and 25% TMAH in H_2O), were evaluated in this work. About 100 mg of TORT-2 CRM, lobster hepatopancreas, was suspended in 4 mL of extractant and the enriched $Me^{201}Hg$ and ^{199}Hg spikes added. The sample and spike were equilibrated by shaking for 15 minutes, followed by UAE for 30 minutes at a constant temperature of 50 °C. After the UAE procedure, samples were

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centrifuged and a 1 mL aliquot removed for Hg species derivatisation with 0.4 mL $NaPr_4B$ (2% in 0.2 mol L^{-1} NaOH), 4 mL 1.1 mol L^{-1} sodium acetate adjusted to pH 5 and 1 mL of hexane with gentle shaking for 10 minutes. The total Hg concentration in each extract was determined by a combustion, trap and purge direct mercury analyser using AA detection whilst the individual Hg species were quantified by GC-ICP-MS. For the total Hg measurements quantitative recovery was only achieved with 5 mol L^{-1} HCl (>98%) or 25% TMAH in H_2O (101%) with lower values obtained for 60 mmol L^{-1} HCL in saturated NaCl (24%) and 3.0 mol L^{-1} KOH in MeOH (83%). However, recoveries of >98% were obtained for iHg and MeHg with all extractant solutions for ss-IDA, again demonstrating that complete equilibration of the added spike with the sample, rather than complete analyte extraction, is necessary to achieve accurate results. No significant methylation of iHg was observed during these analyses but significant demethylation was observed for all extractants except for 5 mol L^{-1} HCl. It should be noted that the double spike procedure employed was able to correct for the observed demethylation of MeHg and thus provided accurate results. The time required for the UAE procedure with 5 mol L^{-1} HCl was also optimised and recoveries of >98%, for both total Hg and ss-IDA, were obtained for three different extraction times of 5, 15 and 60 minutes. Finally, the optimised procedure, UAE for 5 minutes with 5 mol L^{-1} HCl at 50 °C, was applied to both fresh and dried swordfish and shark samples. The values reported for iHg, were <0.02 mg kg^{-1} in both sample types. The results for MeHg were 0.69 and 1.7 mg Hg kg^{-1} for swordfish and shark, respectively. The sum content of the species found were in good agreement with the total Hg concentration determined by AAS. In addition, no differences in the measured total Hg, iHg and MeHg concentrations were observed between the fresh and dried samples for each fish species. However, demethylation of up to 5% of the total MeHg present was observed in these fish samples which was greater than that for the TORT-2 CRM which exhibited <1% demethylation. This latter finding was attributed to the differences in sample preparation for the CRM and the real fish samples, with the former undergoing a procedure to remove natural oils present. This highlights the need for method optimisation on real samples and not just for a CRM. In summary, these two papers are recommended as reference works for the determination and validation of methodologies for Hg speciation in seafoods.

The *photochemical alkylation of iHg* in the presence of ketones, aldehydes and low molecular weight organic acids as alkyl donors has also been investigated.[202] The photochemical experiments were conducted using 4.5 m of 0.8 mm i.d PTFE tubing wrapped around a low pressure Hg vapour UV lamp. The mixtures of iHg, 500 μg L^{-1}, and the alkyl donor, 1 mM, were pumped through the reactor at different flow rates to vary the irradiation time. The reactor effluent was directly analysed by HPLC-AFS and HPLC-ICP-MS and, after aqueous ethylation and/or phenylation, by SPME-GC-MS and purge and trap GC-ICP-MS. Further analyses using electron paramagnetic resonance spectrometry CE-UV and direct scanning UV were also used to identify the reaction radicals and products and to elucidate the photalkylation mechanism. Full details of all the analytical techniques used were given as ESI. When 2-pentanone, 3-pentanone and propionic acid were used as the alkyl donors, unidentified Hg compounds were observed in the HPLC-AFS/ICP-MS chromatograms. These compounds eluted before the MeHg peak and it was postulated that these species contained hydrophilic functional groups produced from the UV induced breakdown of the alkyl donor species. The degree of Hg^{II} alkylation was found to follow the order: ketones > aldehydes > low-molecular-weight organic acids > alcohols. The proposed alkylation mechanism is the formation of a UV energy absorbing Hg^{II}/alkyl donor complex followed by an inter-complex transfer of an alkyl group resulting in the formation of alkylHg species. Due to other photochemical processes, dealklyation of MeHg and EtHg, reduction of Hg^{II} and oxidation of Hg^0 was also observed. As the authors point out, the "the UV irradiation used throughout the photoalkylation experiments is different from natural solar irradiation. Further studies are still needed to validate the possibility of photochemical alkylation of Hg^{2+} under natural sunlight".

Mercury species have also been determined in a variety of *biotic sample types*. A survey of six different marine fish frequently consumed in China has been carried out and their total and MeHg content assessed.[203] Mercury species were extracted from 2 g of fish muscle tissue with 10 mL of 5 mol L^{-1} HCl and 1 g of L-cysteine with closed vessel microwave irradiation at 60 °C for 10 minutes followed by centrifugation and filtration of the extracts. An HPLC-CV-AFS system was used for mercury species quantification. The HPLC mobile phase contained 1 g L^{-1} L-cysteine in 0.06 mol L^{-1} ammonium acetate and a good separation of iHg and MeHg was obtained in six minutes with a RP C18 column. The L-cysteine in the mobile phase was also used as the oxidant for MeHg prior to reduction to Hg^0 with KBH_4 for CV detection. The validity of this approach has been questioned and is discussed further in Section 3.2. The method was validated with two CRMs, DORM-2 dogfish muscle and GBW 10029 tuna fish muscle with obtained MeHg recoveries of 98% for each CRM. The MeHg content of the fish samples ranged from 53 ± 5 ng g^{-1} for Pomfret to 158 ± 8 ng g^{-1} for hairtail. A method for Hg speciation by CE-ICP-MS has also been developed.[204] Under the optimal CE conditions, 50 mmol L^{-1} H_3BO_3 and 12.5 mmol L^{-1} $Na_2B_4O_7$ (pH 9.20) as the buffer solution, +18 kV as the applied separation voltage, a sample injection time of 10 s and a 75 μm i.d 365 μm o.d 85 cm long capillary, EtHg, MeHg and iHg were separated in 25 minutes with detection limits of 0.032, 0.021 and 0.027 ng mL^{-1}, respectively. The method was subsequently applied to river water and dried fish samples. No mercury species were detected in the river waters. Only MeHg was found in the dried fish samples at a concentration of 1.2 μg Hg g^{-1}. A preliminary study has been undertaken to assess the correlation between fish consumption and the concentration of MeHg in human fingernails.[205] Complete dissolution of the nail clippings, using 50 mg of sample in 5 mL of TMAH, was achieved with shaking for 12 hours. After dilution of the extracts to 50 mL, mercury species were derivatised in a 25 mL aliquot with the addition of 3 mL of 1% $NeBEt_4$, 8 mL of a buffer solution (final pH not stated), 3 mL of iso-octane, containing dibutyl-dipentyltin at

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concentration of 2% as an internal standard, and shaking for 30 minutes followed by the addition of a further 1 mL of 1% $NaBEt_4$ and shaking for a further 30 minutes. After centrifugation, an aliquot of the iso-octane layer was injected into a GC-ICP-MS system for separation and quantification of the mercury species present in 8 minutes. A GC carrier gas, consisting of He with 0.1% Xe and flowing at 6.5 mL min^{-1}, was used both to optimise the ICP-MS conditions and also act as an internal standard to correct for instrumental drift. A tuna fish CRM, BCR 463, which as the authors point out is not ideal, was subjected to the same extraction/derivatisation procedure as the nail clippings and a recovery of 99 $\pm$ 4% was obtained for MeHg. The MeHg content in fingernail samples obtained from two Brazilian fisherman and from one "Dutch vegetarian with fish consumption" (so strictly not a vegetarian) were of the order of 5 ng g^{-1}. This was found to be "of significance" when compared to the concentrations found in samples taken from a "Brazil reference person" and a "Dutch vegetarian without fish consumption", of 2 ng g^{-1} and 0.5 ng g^{-1}, respectively. The authors indicated that a further, more comprehensive study is now needed to confirm these initial results. Concern for the welfare of *Ursus maritimus* has led to a study of the mercury content and speciation in polar bear brain tissue samples.[206] A freeze dried brain tissue sample (0.1 g) was subjected to a UAE procedure for 30 minutes at 35 °C in 5 mL of an extraction solution comprising 0.1% HCl v/v, 0.1% 2-mercaptoethanol v/v and 0.15% KCl v/v. After 30 minutes the extracts were centrifuged, the supernatant was removed and the solid residue further extracted as before. Subsequently, the extractions were combined, centrifuged again and the supernatant collected, filtered and made up to 10 mL. An RP C18 column was used to separate the extracted Hg species and these were detected by ICP-MS by monitoring the ^{202}Hg signal. The CRM used to assess the method accuracy was DOLT-4 and the recovery achieved was equivalent to 102% of the certified value of 1.33 mg kg^{-1}. Unusually for a mammal, no iHg was detected in the polar bear samples with the MeHg content found to be 0.12 mg kg^{-1} in the brain stem, 0.23 mg kg^{-1} in the cerebellum and 0.28 mg kg^{-1} in the frontal lobe. The proportion of Hg detected in the brain compared to previously reported liver Hg concentrations was also unusually low and is a possible indicator of an as yet unknown 'MeHg transport restriction mechanism' in polar bears. A GC-pyrolosis-AFS method was also developed for Hg speciation measurements in red deer and wild boar samples.[207] An MAE procedure, utilising TMAH as the extractant, was used to solubilise Hg species present in freeze dried field collected liver and kidney samples. After extraction, mercury species were derivatised with $NBEt_4$ at a pH of 3.9 followed by head space SPME *via* a PDMS fibre prior to desorption in the GC inlet port. Two CRMs, NCS-ZC 71001 (beef liver) and BCR 186 (pig kidney), were also subjected to the analytical procedure. These two CRMs were certified for total Hg only but a sum of the species approach, with only iHg and MeHg being detected, resulted in total Hg values in good agreement with the certified values (0.173 $\pm$ 0.035 compared with 0.18 mg kg^{-1} for NCS ZC 71001 and 2.160 $\pm$ 0.057 compared with 1.97 $\pm$ 0.04 mg kg^{-1} for BCR 186). Detection limits were reported as 0.17 μg L^{-1} for MeHg (as Hg) and 0.28 μg L^{-1} for iHg in the extract solutions which translates to 32 ng g^{-1} and 53 ng g^{-1} for MeHg and iHg respectively for liver tissues and 35 ng g^{-1} and 58 ng g^{-1} for MeHg and iHg respectively for kidney tissues. The highest MeHg concentration was found in red deer liver 329 and 425 ng g^{-1} as Hg for the two samples analysed with MeHg being found in only one kidney sample at 78 ng g^{-1} as Hg. The iHg concentrations were greater in the kidney samples than the liver samples with a maximum observed value of 740 ng g^{-1}.

Mercury species have been quantified in sediments and waters as part of a study of the impact of anthropogenic and hydrological constraints on the biological functioning of the Bach Dang estuarine ecosystem in North Vietnam.[208] The water samples taken were separated into a "dissolved" fraction, which includes colloidal material and suspended particulate matter (SPM) by passage through a 0.45 μm PVDF filter membrane. Sediment samples were lyophilised and crushed but the final size fraction used was not stated. Mercury species in the SPM and sediment samples were extracted, after spiking and a 15 minutes equilibration period, using a focussed MAE procedure at 75 °C for four minutes. The species present in the samples were quantified by ID-GC-ICP-MS after spiking with ^{199}Hg enriched (91%) iHg and ^{201}Hg enriched (96.5%) MeHg. The propylation procedure for Hg species and GC-ICP-MS separation was the same as that described above in ref. 200. Two CRMs were used to monitor the accuracy and precision of the method, BCR-579 coastal seawater, for total Hg in water samples, and IAEA 405 estuarine sediment for mercury speciation in the SPM and sediments. The results of the CRM analyses, for triplicate determinations in each case and given as the percent deviation from the certified value and an RSD% value respectively, were total Hg in BCR-579 2.1% and 0.8%, 3.5% and 5.4% for MeHg in IAEA 405, 3.4% and 3.8% for total Hg in IAEA 405 and 3.7% and 2.2% for TBT in PACS-2. For the actual samples the total Hg content ranged from 0.2 to 1.6 ng L^{-1} in the dissolved phase and 68 to 198 ng g^{-1} for the SPM with MeHg ranging from 0.01 to 0.03 ng L^{-1} and 0.3 to 2.1 ng g^{-1} for the same two phases whilst sedimentary concentrations ($n = 4$) were total Hg 72 ng g^{-1} and MeHg 0.23 ng g^{-1}. It was concluded that iHg was the major mercury species entering the Bach Dang estuary, being transported downstream bound to SPM and that iHg methylation increased as SPM residence time increased. When flux values were taken into account the estuary appeared to be an important source of iHg in the wet season and MeHg in the dry season to the surrounding coastal zone. An alternate approach to the determination of mercury species in seawaters has been described in which a sulfonic acid based cation exchange column was inserted in the sample loop of an HPLC-ICP-MS system.[209] Mercury species were extracted onto the column (50 mm × 4 mm i.d.) from 30 mL of filtered (0.2 μm) seawater flowing at 9 mL min^{-1}. This was followed by a wash step, with water for 1 minute at the same flow rate, to remove the seawater matrix ions. The species were eluted from this column using 4% MeOH and 10 mM L-cysteine at pH 8 flowing at 1 mL min^{-1} which was also the optimal mobile phase for the subsequent separation by HPLC, achieved using an RP C18 column (50 mm × 4.6 mm i.d, 5 μm) coupled directly to the ICP-MS instrument. Under these conditions Hg^{II}, MeHg and EtHg

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were separated in under three minutes, although the separation of the first two species was barely baseline resolved giving a total time per sample of 7.5 minutes for the whole procedure. The measured contents of MeHg (0.34 ± 0.02 ng mL^{-1}) and iHg (0.63 ± 0.04 ng mL^{-1}) in a seawater CRM, GBW (E) 080042, were in aggregate in agreement with the certified total Hg value of 1.00 ± 0.06 ng mL^{-1}. Limits of detection of 0.042, 0.016 and 0.008 ng L^{-1} were obtained for iHg, MeHg and EtHg, respectively, and a pre-concentration factor of 1250 was reported. The method was applied to seawater samples from three Chinese cities with the iHg concentrations ranging from 1.2 to 2.4 ng L^{-1}, the MeHg concentrations varied between 4.4 and 68 ng L^{-1} with the EtHg concentration range being 0.58 to 0.93 ng L^{-1}. These results, especially for MeHg, indicate a more polluted system than that of the Bach Dang estuary example described earlier.

Two papers report on the *association of Hg to proteins*. One paper investigated the binding of MeHg with proteins using BCR CRM 464 Tuna fish muscle as the model system.[210] Four different extraction procedures, three utilising TRIS buffer and one involving 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), were evaluated. The optimal extraction procedure was found to be 4% SDS and 30 mM TRIS at pH 8.0 for 14 h at 37 °C which gave a recovery for Hg of $98.1 \pm 3.4\%$. Recoveries of greater than 110% were observed with the procedure using CHAPS which was attributed to contamination of the reagents. Quantification of Hg in the extracts was by ID-ICP-MS using a spike enriched in ^{200}Hg. Subsequently, SEC-ICP-MS studies revealed that the MeHg was only bound to a protein fraction with a molecular mass of >70 kDa. An SDS-PAGE separation followed by excision and digestion of the protein spots with nitric acid was then performed. Analysis of these digests showed that the majority of the Hg present was in a fraction with a RMM of approximately 220 kDa. Finally, protein spots from a separate SDS-PAGE separation were enzymatically digested, with trypsin in a multistage procedure, followed by analysis with HPLC-ICP-MS, which identified at least five Hg containing peptides. Twenty eight peptides were identified in total by HPLC-ES-MS-MS and database searching suggested that the original protein to which the MeHg was bound belonged to the myosin family. A simple aqueous UAE procedure, 30 s at 100 W, followed by HILIC coupled with ICP-MS or ES-MS-MS, has been used to identify HgMT complexes in homogenised white dolphin liver.[211] A gradient elution profile, with acetonitrile and 5 mM ammonium acetate at pH 5 as the two components with a combined flow rate of 50 μL min^{-1}, was used for the HILIC separations. The HILIC-ICP-MS chromatograms showed a "fairly large peak" followed by a less intense peak eluting 1.3 minutes later which each contained Hg. The MT isoforms were subsequently identified, from the HILIC-ES-MS-MS data, as MT2–Zn_6Hg.

5.8 Selenium

Selenium continues to be one of the more popular elements for speciation studies. Applications focusing on the determination of Se species (seleno amino acids, selenometabolites, selenoproteins and selenium-containing proteins) in biological samples (food, plants and animal tissues, biological fluids, microorganisms) by using multidimensional approaches (HPLC-ICP-MS, HPLC-ESI-MS(MS) and LA-ICP-MS based techniques) constituted most of the published work. Of particular interest this year, at least in terms of number of publications, would seem to be the application of XRF and XANES for mapping and quantifying Se in biological tissues as well as the use of selenium isotopes as tracers to investigate Se metabolisms in animal and plants. Selenoproteins and SeMet quantification in serum and plasma continues to be a very active topic, especially for developing CRMs as valuable tools for establishing selenium status in human plasma.

During the past year, *two reviews* have appeared. A comprehensive review of Se bioaccessibility in food by Thiry *et al.*[212] summarises recently published data concerning both Se speciation and Se relative bioaccessibility in various foodstuffs, and discusses the latter in terms of species-dependent metabolism in humans. In a review in Japanese, Ogra[213] focus on two topics: nano-speciation, by using multi-mode gel filtration capillary HPLC, and selenometabolomics.

Studies on the conversions and transformations of Se in plants by HPLC-ICP-MS and HPLC-ESI-MS-MS have been a topic of much research in the year under the review. Selenosugars in edible plants (wheat, rice and maize) were identified for first time[214] using cation-exchange (150 mm × 2.1 mm, 10 μm) HPLC-ICP-MS and ES-Orbitrap MS/MS. The occurrence of Se-containing compounds was dependent on the type of crop: Se^{IV} and SeMet (or oxidised SeMet) were detected in all the samples whilst MeSeCys was detected in wheat but not in maize or rice. Interestingly, other previously unreported compounds were identified from the Se isotopic pattern. A m/z 345 compound was identified as 2,3-dihydroxypropionyl-selenolanthionine and others present were identified as Se-containing monosaccharides (hexose moiety, m/z 317 and m/z 358) or Se-containing disaccharides (hexose–pentose moiety, m/z 407 and m/z 408). The results were validated by HILIC (250 mm × 1 mm, 5 μm) combined with ES-Orbitrap MS/MS. Enzymatic glycosylation and/or chemical reaction among sugars and selenium were addressed by the researchers as the origin of these compounds in plants.

Mechora *et al.*[215] performed selenium speciation, by on-line coupling of HPLC-ICP-MS following enzymatic hydrolysis with protease, and the method was applied to Se^{VI}-enriched cabbage. Cation-exchange column (4.6 mm × 250 mm × 5 μm) and AEC (4.1 mm × 250 mm × 10 μm) were used for Se species separation. The concentrations of Se in cabbage plants foliarly sprayed twice with Se^{VI} at 20 mg L^{-1} level were in the range from 4.77 μg g^{-1} in leaves to 5.51 μg g^{-1} (DM) in roots. The main Se species in cabbage was SeMet representing 17% of total selenium. A proper mass balance was included and the results were validated by applying the method to a CRM (Durum Wheat Flour NIST RM 8436). One of the novelties of this year has been the use of selenium isotopes as tracers to investigate Se metabolism in plants, and also to achieve rapid speciation analyses and quantification of Se by HPLC-ICP-MS. Following this approach,[216] an acetone extract of selenised garlic was spiked with a mixture of labelled standards synthesised and purified by

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the authors ($Me^{76}SeCys$, ^{77}Se-sugar, ^{76}Se-methylselenonic acid, ^{77}Se-methylseleninic acid, $^{77}SeMet$, $^{78}Se^{VI}$ and $^{82}Se^{IV}$). Subsequently, the spiked extracts were analysed by reverse phase chromatography coupled to an ICP MS with an octopole reaction cell. Deuterium was used as reaction gas, to avoid the formation of ^{81}BrH, although SeD polyatomic species were detected and corrected for mathematically. Quantitative determination of Se compounds was performed by standard addition in $^{76,77,80}Se$ and the concentrations of γ-Glu-MeSeCys, MeSeCys, and SeMet in the sample were calculated to be 724, 415, and 311 ng mL^{-1}, respectively, corresponding to the 79.5% of the total selenium in the selenised garlic extract. The method was also applied to yeast and urine. In another paper[217] enriched and intrinsic labelling of onions and carrots was performed by foliar spraying of $^{77}Se^{IV}$. The cation-exchange (100 mm × 3.0 mm, 5 μm)-HPLC-ICP-MS analysis of the proteolytic plant extracts show that γ-Glu-$Me^{77}SeCys$ and $Me^{77}SeCys$ were detected in onion bulbs while $Me^{77}SeCys$ and $^{77}SeMet$ were the predominant Se-species in onion leaves. Surprisingly, γ-Glu-$Me^{77}SeCys$ was absent in onion leaves whilst $Me^{77}SeCys$ was detected in the ^{77}Se-fortified carrots by HPLC-ICP-MS and its identity verified by ESI-MS-MS. Quantitative analysis of Se species showed that the majority of the applied $^{77}Se^{IV}$ in onion and carrots was detected as organic Se species.

Several attempts to identify *selenium-containing proteins and selenopeptides in plants* have been reported. Three major selenium-containing proteins were isolated from Se-enriched brown rice.[218] Protein purification was achieved using Sephadex G-100 gel filtration and diethylaminoethyl cellulose Sepharose fast flow AEC. The molecular weights of the resulting three selenium-containing protein fractions were of 15 kDa, as determined by SDS-PAGE and MALDI-TOF-MS. The proteins showed strong antioxidant activities when applying three different free radical-scavenging assays: the hydroxyl radical-, superoxide anion-, and 1,1-diphenyl-2-picrylhydrazyl-scavenging assays. Unfortunately, the chemical structure of the Se-containing proteins was not possible to be determined by MALDI-TOF-MS. Proteins containing selenium has also been identified in beans of Se-enriched soybeans plants,[219] after extraction the total Se in the dissolved proteins was 75 ± 5 μg g^{-1}. Subsequently, proteins were screened by a two-dimensional chromatography method involving SEC (7.5 × 300 mm, 10 μm) and AEC (5 × 50 mm, 10 μm). Selenium containing AEC fractions were digested with trypsin for analysis by HPLC-Chip-ES-MS. Two main categories of proteins, maturation proteins and protease inhibitors, were found in Se-containing fractions. Identification of Se-containing peptides was performed by assuming their co-existence with the corresponding sulfur analogues. Based on that premise, the Se-containing peptide KSDQSSSYDDDEYSKPCCDLCMCTRS, part of the sequence of protein Bowman–Birk proteinase isoinhibitor (glycine max), was found in one of the Se-containing fractions. Both of these two articles highlight the difficulties in detecting selenopeptides in vegetables tissues.

The variety of analytical techniques used for selenium speciation in plants is still increasing. A good example of this is the combined used of ICP-MS, μXRF mapping, μXANES, bulk XANES to map the localisation of Se, and HPLC-ICP-MS and to quantify different Se forms, in Se enriched-Brassica seeds and seed meal.[220] Micro-XRF elemental mapping showed that selenium was mainly located in cotyledons and roots of seed embryos. The application of microfocused Se K-edge XANES, bulk XANES and HPLC-ICP-MS confirmed the presence of SeMet and MeSeCys. Similarly, XRF mapping and XANES were used to characterise Se distribution and speciation in all organs of *Astragalus bisulcatus* collected in natural seleniferous habitat.[221] The study was also applied in a novel way to microbial symbionts and herbivores associated with the plants. It was reported that Se was present in all organs, mainly as organic C–Se–C compounds (MeSeCys). Two Se-resistant herbivorous moths were discovered on *A. bisulcatus*. Adult moths, larvae, and wasps all accumulated predominantly C–Se–C compounds.

Two papers described an investigation *of the bioaccessibility of selenium from different soils*. An *in vitro* Physiologically Based Extraction Test (PBET) and a phosphate buffer extraction were applied to two soil CRMs (Resource Technology Corporation CRM023-050 and CRM025-050) and two soils from California (R1 and R2).[222] Both HPLC-ICP-MS and HPLC-HG-AFS were employed to analyse the extracts. The gastrointestinal PBET stage provided mean bioaccessible values of 99% for CRM023-050, 78% for CRM025-50, 61% soil R1 and 57% for soil R3. In the PBET extracts only Se^{IV} was found for the two CRMs whilst for the Californian R1 and R3 soils Se^{VI} comprised 58 and 70% of the total Se respectively. The only data given for the phosphoric acid extractions is that the correlation coefficients (95% confidence interval) were greater than 0.99 when the data was compared to the PBET values for both Se^{IV} and Se^{VI}. In the second paper[223] agricultural soils amended with 20 mg kg^{-1} of Se^{IV} and Se^{VI} were analysed for extractable Se using 0.016 M KH_2PO_4 and AEC-ICP-MS. The stability of Se species in the phosphate extracts was variable, depending on temperature and storage time and immediate (<1 h) analysis of the soil extracts was recommended. Recoveries were 90% and 18.5% to 46.1%, for Se^{VI} and Se^{IV}, respectively. Extractable Se values ranged between 6 and 13 μg kg^{-1} for six UK arable soils with Se^{IV} as the major Se specie. Moreover, oxidation of the phosphate extracts with H_2O_2 showed the presence of unidentified organic Se of microbial or plant origin.

Studies of interconversion and transformation of Se compounds by microorganisms is still of interest this year. These studies can be divided in two types, one devoted to Se-metabolites in yeast and the other focused in the use of microorganism for preparing Se-enriched food and related issues. Lobinski's group[224] continuous to investigate Se metabolites in Se-yeast by applying an Orbitrap as a mass analyser. This approach offers low μg L^{-1} detection and good resolution. The instrumental set-up was based on parallel coupling of 2D SEC-RP-HPLC with diode array UV, ICP-MS and ES-LTQ-Orbitrap-MS detection. Its application allowed the identification of the largest number of metabolites (49) in Se-yeast aqueous extract reported so far. Optimal separation conditions were applied by using a miniaturised SEC column (4.6 mm × 300 mm, 4 μm) with flow-rates down to 300 μL min^{-1} followed by a second dimension ultra-high performance C_{18}-RP column (1.0 mm × 100 mm, 1.8 μm)

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without using an organic modifier. Concerning to the second type of studies, the production of Se-metabolites by *Chlorella sorokiniana* grown in a Na_2SeO_4-enriched medium was explored for preparing Se-enriched food.[225] Selenium species in enzymatic hydrolysed extracts of alga were determined by HPLC-ICP-MS. The instrumental coupling was based on a bidimensional chromatographic separation, by in series switching of a C_{18} RP (250 mm × 4.60 mm, 5 μm) column and a chiral column (250 mm × 4.6 mm, 5 μm), allowing separation of L-SeMet and D-SeMet. Other species found were $SeCyst_2$, and SeMeSeCyst. The SeMet concentration in the algae increased from about 7 to 15 μg g^{-1} and Se^{VI} from 15 to 25 μg g^{-1}, after 500 h of exposure. Selenium biotransformation during brewing by using *Saccharomyces uvarum* and *S. cerevisiae* for ale and lager fermentation was evaluated by Sanchez-Martinez *et al.*[226] The percentage of selenium incorporated into beer, added at concentrations of between 1.0 and 10 μg mL^{-1} to the brewing mixture, was 55 to 60% of the selenium initially present. The main compound identified, by AEC-ICP-MS following ultrasonic assisted enzymatic hydrolysis and dialysis, in yeast and beer was SeMet. The results from Se-beer demonstrated that SeMet is randomly oxidised to SeMetO making it difficult to achieve a proper mass balance. The biotransformation of Se-compounds in gut by probiotic bacteria was evaluated by both *in vitro* and *in vivo* studies.[227] For the former, SeMet and Se^{IV}, commonly present in Se-supplements, were anaerobically incubated for 24 h at 37 °C in the presence of antibiotic-resistant probiotic strains (*Streptococcus salivarius K12, Lactobacillus rhamnosus 67B, Lactobacillus acidophilus L10, and Bifidobacterium lactis LAFTI® B94*). Both SeMet and Se^{IV} were metabolised to dimethyldiselenide (DMDSe) and dimethylselenide (DMSe) and Se^{IV} was also converted to elemental selenium. Volatile selenium compounds and SeMet were measured by GC-MS and HPCL-AFS, respectively. In the *in vivo study*, treatment with the probiotic mixture significantly increased Se levels in the liver and decreased them in the kidney after administration of SeMet compared to untreated rats.

A number of papers cover *selenium speciation in fish and mammals tissues*. The biological role of Se metabolite, Selenoneine (2-selenyl-*N*,*N*,*N*-trimethyl-L-histidine) in preventing toxicity against iHg and MeHg was studied by Ogra *et al.*[228] An *in vitro* assay was performed by incubating liver homogenates of hawksbill turtle (as a source of selenoneine) with iHg and MeHg during 1 hour at 37 °C in the absence and in the presence of exogenous ^{82}Se labelled-selenite. Once incubated, the homogenates were analysed by a multimode gel filtration column (7.5 × 300 mm) coupled to ICP-MS used in the D_2 reaction mode. The distribution of endogenous selenium and exogenous mercury in the liver cytosol showed that selenoneine seems to be not involved in the detoxification of mercury whereas Se^{IV} reacted with iHg after being converted into selenide by GPx. In a different study,[229] a water soluble selenoprotein isolated from *Misgurnus anguillicaudatus* tissue was investigated. The trypsin/Flavourzyme combination was used to hydrolyse the *M. anguillicaudatus* selenoprotein for further analysis by RP-HPLC-ICP. The two major species found in the hydrolisates were SeMet and $SeCyst_2$. When analysing the enzyme-hydrolised samples by HPCL-ESI-MS-MS, an unknown Se-containing peak with a similar polarity to $SeCyst_2$ and peaks likely to be due SeMet-containing peptides were discovered. The SeMet concentration in the enzymatic extracts were as high as 6.28 mg kg^{-1} and the authors suggest *M. anguillicaudatus* hydrolisates as a good source for preparing Se-enriched supplements. Similarly, Niboshi (a processed Japanese anchovy, *Engralus japonicus*) extract was characterised for preparing Se-supplements.[230] Polar solvents (water and ethanol) were selected for extracting Se-species from Niboshi. Only 6 to 12% of the total selenium in the Niboshi was extracted using these solvents. Around 90% of selenium in the extracts was present as low molecular mass organoselenium compounds (<5 kDa), including seleno amino acids and derivatives. Selenium fractionation, by sequential separation with AEC (Q-Sepharose in OH form and SP Sepharose in H form columns) revealed that most of the selenium present in the Niboshi has anionic and/or amphoteric characteristics. Different workers studied Se speciation in tissues of female turkeys[231] supplemented with varying proportions of selenised-enriched yeast or sodium selenite in a study involving 216 animals. Important differences between tissue types and treatments in the distribution of SeMet and SeCys, and the activity of tissue and erythrocyte GSH-Px were observed. The SeCys was the predominant form of Se in visceral tissue and SeMet the predominant one in breast tissue. Se accumulation was higher in those birds supplemented with selenised-yeast. According to the authors, meat quality was not affected by Se supplementation. The localisation of selenium in mouse liver and kidney[232] has been explored by SR-XRF. Selenium was found to be homogeneously distributed in liver whereas in kidney Se was highly localised as circular structures surrounding the proximal tubes. The location of selenium at the basement membrane of kidneys was associated with GPx3, evidencing the potential of XRF for elucidation of selenium metabolic pathways and distribution in mammals at submicron resolution.

Methods for the *determination of selenium in various body fluids* have also been developed in the period covered by this Update. A fast method[233] has been developed for Se, Cu, Fe and Zn speciation in human serum. It was based on the use of monolithic anion exchange micro-columns (1 × 50 mm) coupled to ICP-MS. In contrast with traditional AEC, the new column required serum volumes as low as 1 mL and it provided six-fold faster separation (10 minutes) while maintaining chromatographic resolution. The method was applied to easily detect quantitative and qualitative differences between RMs (Seronorm L1 and Seronorm L2) and among serum, heparinised plasma and EDTA-treated plasma. Unlike Fe, Cu and Zn, Selenium chromatographic profiles were not affected by EDTA because this chelator is unable to break the Se–C bounds. A combination of species unspecific IDA affinity LC-ICP-MS, LA-ICP-MS, and ES-MS-MS was developed to identify and quantify SelP, GPX3 and SeAlb in a human plasma reference material SRM 1950.[234] Only two selenium-containing proteins, albumin and SelP, were detected after applying a shot-gun proteomic approach to three selenium-containing fractions, corresponding to the non-retained fraction (F1), the heparin-Sepharose retained fraction

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(F2), and the blue-Sepharose retained fraction (F3). However SelP identification in F2 and F3 fractions was not conclusive. Unambiguous identification of SelP and GPX3 was achieved by applying LA-ICP-MS followed ES-MS-MS. For this purpose, proteins in affinity fractions were separated by GE, electroblotted onto a PVDF membrane, and Se was detected by LA-ICP-MS analyses. Proteins in Se-containing bands were digested with trypsin and then identified by ES-MS-MS. As a result of this research, SRM 1950 is reported to be the first human plasma SRM with a measured total Se value of 105.5 ± 2.3 ng g^{-1} and mass fraction values of 23.6 ± 1.3 ng g^{-1} as Se for GPx3, 50.2 ± 4.3 ng g^{-1} as Se for SelP, and 28.2 ± 2.6 ng g^{-1} as Se for Alb. A similar LA-ICP-MS based technique was applied for detecting selenoproteins in polyacrylamide gel and PVDF membrane after blotting.[235] Parameter optimisation was achieved by using two in-house reference samples consisting of a GPx band in the gel and on the polymer, respectively, and two laser systems: a 213 nm laser and a 1030 nm high repetition rate femtosecond laser with galvanometric optics. The study allowed for the first time LA-ICP-MS detection of selenoproteins in human cell extracts with the selenium concentration at the 10 ng mL^{-1} level. Suzuki *et al.*[236] developed a method combining heparin AF and SEC-HPLC coupled to ICP-MS to determine selenoproteins in human and mouse plasma. The instrumental set-up was appropriated for determining the Se concentrations incorporated in Sel-P in mouse plasma, but not in human plasma, because of nonspecific adsorption of plasma-extracellular glutathione peroxidase (eGPx) and albumin on the heparin AF column.

Dernovics *et al.*[237] applied a liquid phase peptide synthesis protocol to make a *sec(2)-containing oligopeptide*, (Boc-GGFG)-*sec*(2)-(Boc-GGFG) (*m/z* 1173.3, [M + H](+)), that was subsequently used for method evaluation purposes in selenium speciation The comparison of two sample preparation techniques, *i.e.* methanesulphonic acid (MSA) based digestion and proteolytic digestion with protease XIV, on the SeCys residue integrity demonstrated that the use of MSA resulted in the decomposition of SeCys even after derivatisation with iodoacetamide.

Identification and quantification of *Se metabolites in urine* has been reported in several papers. Lu *et al.*[238] has utilised HPLC-ES-MS/MS to study Se in urine. In this case trimethylselenomium ion ($TMSe^+$), and SeMet and the two selenosugars, methyl 2-acetamido-2-deoxy-1-seleno-β-δ-galactos-glucos-amine (SeGalNAc) and (SeGluNAc), respectively were detected in urine samples. Separation of Se-compounds was achieved by HPLC with a combination of a cation-exchange pre-column (20 mm × 4.6 mm) with a RP column (150 mm × 4.6 mm) connected in tandem, and a mobile phase consisting of 60 mM ammonium acetate at pH 6.36 with 2.5% ACN. Both standard addition and an isotopically labelled internal standard (^{82}SeGalNAc as internal standard for SeGalNAc) were used as approaches for minimising non-spectral interferences. The method provided LODs of 0.3–0.6 μg L^{-1} for $TMSe^+$, 1.5–5.6 μg L^{-1} for SeMet, 0.1–0.6 μg L^{-1} for SeGalNAc and 0.01–0.03 μg L^{-1} for SeGluNAc. The technique was sensitive enough to detect and quantify SeGalNAc and $TMSe^+$ in urine of two volunteers prior to and after Se supplementation. SeMet was not detected as a urine excretory Se metabolite. In addition, $TMSe^+$ was characterised for the first time by ES-MS-MS in urine. A rapid method for simultaneous identification and quantification of Se metabolites in rat urine using multiple standards labelled with different isotopes was reported by Ohta *et al.*[216] The method utilised HPCL-ICP-MS for analysing the urine samples after single administration of ^{76}Se-methylselenonic acid ^{77}Se-methylseleninic acid, $^{78}Se^{VI}$ and $^{82}Se^{IV}$. Separation and determination of Se metabolites was performed with a gel filtration column (150 × 4.6 mm, 3 μm) coupled to ICP-MS. The isotopically labelled standards enabled the identification of several Se-compounds by a simple speciation procedure. Methylselenonic acid, Se^{VI}, selenosugar, and $TMeSe^+$ ions were found in the urine samples. Momomethylated selenium compounds were generally excreted as $TMeSe^+$ rather than iSe whilst Se^{VI} was excreted almost unaltered. The urine of seven healthy volunteers,[239] orally supplemented with aqueous solution containing $^{82}Se^{IV}$ and $^{82}Se^{VI}$, has been analysed by HPLC-ICP-MS. Two chromatographic separation mechanisms involving AEC (250 × 2 mm) and RP (100 mm × 2 mm, 3 μm) were used for selenium speciation and selenosugar quantification, respectively. The mean total urinary excretion of ^{82}Se following $^{82}Se^{VI}$ administration was 34% while the mean total excretion of ^{82}Se after $^{82}Se^{IV}$-administration was 3.2% of the ingested amount. The results again evidence different human metabolic pathways for Se^{IV} and Se^{VI} of which Se^{VI} was excreted almost unaltered while Se^{IV} was primarily excreted as selenosugar (SeGalNAc). Finally, a new method[240] using a sulfonated polystyrene–titania coated stir bar sorptive extraction and HPLC-ICP-MS was established for the determination of seleno amino acids and seleno-oligopeptides in human urine and garlic. The mechanism was based on a cation exchange interaction, allowing the extraction of high polarity seleno-amino acids and seleno-oligopeptides in biological samples without derivatisation. The LOD of the developed methods for six target selenium species were in the range of 46 to 186 ng L^{-1} (as Se) and with the RSDs varying from 4.9 to 12%. The method was validated using a CRM (SELM-1 selenium enriched yeast).

The measurement of *Se speciation in flue gas desulfurisation (FGD) waters* from coal-fired power plant has been reported in two papers. Selenosulfate and selenocyanate were identified for first time in such waters by AEC-ICP-MS with 100 mM NaOH as eluent.[241] The results were later confirmed by ESI-MS-MS. Aside from Se^{VI} and Se^{IV}, selenosulfonate constituted a major fraction of selenium (up to 63% of the total Se) in some of the waters analysed. The sensitivity of the ESI-MS-MS was not good enough to identify other nine Se-containing peaks also detected in the waters. In another paper, the effect of oxidation conditions on selenium speciation in such waters was evaluated.[242] The Se K-edge XANES spectrum of Se^{IV} was gradually shifted to that of Se^{VI} in the presence of peroxodisulfate which was the dominant oxidising agent in wet flue gas desulfurisation. Good agreement in Se^{VI} and Se^{IV} quantification values were obtained by ICP-MS and XAS.

5.9 Tin

The number of publications reporting studies related to Sn speciation has continued to decline, continuing the trend

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reported in recent reviews.[243,244] There have been no reports of novel studies on either water or sediment samples. However, Campillo *et al.*[245] have reported on the use of headspace solid-phase microextraction for the speciation of 7 organotin compounds (MBT, DBT, TBT, MPhT, DPhT, cyhexatin and fenbutatin oxide) in *honey and wine samples* using GC coupled to MIP-AED and MS detectors. The analytes were derivatised *in situ* with sodium tetraethylborate. A 100 μM polydimethylsiloxane fibre proved the most suitable for preconcentrating the derivatised analytes. When MIP-AED and MS detection were compared, higher sensitivity was attained for all compounds with MS, although MIP-AED offered the advantage of element specific chromatograms. In addition to the poorer S/Ns, the AED system was found to be more expensive to operate in terms of gases and maintenance. Using MS, detection limits ranged from 0.3 to 4.3 pg (Sn) g^{-1}, depending on the compound for honey samples, and from 0.1 to 2 pg (Sn) mL^{-1} for wine samples. The method was applied to eight real honey samples and 21 wine samples. No OTC were found in the honey, but at least one compound was found in each of the wines at concentrations ranging from 0.05 to 5 ng mL^{-1}. Recoveries from spiked wine samples (5 to 170 pg mL^{-1}, depending on the compound) were not significantly different from 100%.

The determination of *TEL and TBT compounds in human urine* by LLE and GC-MIP-AES following extraction and *in situ* derivatisation by tetrapropyl-borate has been reported.[46] The alkylation reaction was done in the aqueous and urine medium and the less-polar derivatives were extracted in hexane. Samples of urine (10 mL) were extracted using 1 mL hexane and an aliquot of 1 μL of extract is injected onto the column. A HP-125 m × 0.32 mm × 0.17 mm capillary column coated with 100% PDMS was used for the separations. Tin was detected at 303.419 nm. The calculated detection limits for TEL and TBT were 0.05 μg Pb L^{-1} and 0.48 μg Sn L^{-1} respectively. The method can be directly applied to both undiluted and diluted urine samples with RSD values of less than 5%.

6 Macromolecular analysis

This section provides details of a range of diverse methods for the analysis of different biomolecules using elemental techniques.

6.1 Metalloproteins, metalloproteomics and metallomics

About one third of all proteins contain a metal ion *in vivo* and about one half of all enzymes require a metal cofactor. However, despite the advances in genomic, proteomic and analytical techniques over the last few years, characterisation of the metalloproteome (identification and quantification of proteins involved in metal homeostasis) in any organism remains incomplete. A very useful review[246] put the techniques, approaches and drawbacks involved in the *fractionation and detection methods used for metalloproteomics* into their current context. The review highlighted the main pitfalls for the unwary when trying to untangle the complexities of the extensive range of metalloproteins found *in vivo*, including: changes in the chemical environment for the proteins during cell lysis; exposure to oxygen, which is a problem for thiol-containing and redox active proteins; and dependence of the metal–ligand interaction on pH and ionic strength. The paper went on to review the main techniques that have been used and which offer the capability to overcome the highlighted problems. These include the broad areas of: 2D gel electrophoresis which separates first by charge (isoelectric focusing) and then by molecular mass; 2D or multidimensional LC using any of the conventional HPLC types such as IE, SEC or RP chromatography in series. The application of immobilised metal affinity chromatography (IMAC) which can be used to "fish out" proteins that contain metal-binding motifs was also covered. The caveat for IMAC is that some proteins may have the capacity to bind metals but never do so *in vivo* and some that avidly bind metals, but are not retained because the binding site is unavailable due to the presence of a strongly bound metal still being present. The different native and non-native forms of molecular mass spectrometry such as ES-MS-MS which is used in conjunction with the aforementioned chromatographic methods are described very nicely in this paper. In a related technical paper[247] the same authors considered the major Co, Fe, Mn and Ni binding proteins in the marine cyanobacterium *Synechococcus* sp. In this instance fractions were collected after separation using GPC followed by SAX using mini-spin columns. The proteins were eluted from the SAX column in 80 μL fractions using a stepwise NaCl gradient of 0.1 to 2.0 M. This approach effectively concentrates the protein during the elution steps. The SAX fractions were further analysed using ICP-MS and fractions giving rise to the most prominent metal peaks were run on SDS-PAGE with Coomassie blue staining to identify the protein bands. These bands were excised from the gel and following in-gel trypsin digestion, the peptide masses were identified by MALDI-TOF-MS. Metal-binding proteins present in soluble fractions of the cell lysate were also isolated using IMAC columns that were charged with Co^{2+}, Fe^{3+}, Mn^{2+} or Ni^{2+} as "capture" ions. Eighteen proteins able to bind the metals investigated were identified using the two separation strategies employed. The main advantages of the protocol used were: preservation of the native protein interactions; no requirement for labelling prior to detection; and rapid analysis compared to 2D GE.

The *speciation of trace elements can provide an insight into human nutrition* both by investigation of the food source but also measurement of the subsequent species present in the body. Investigation of human samples provides information on the distribution of metalloproteins in circulation both due to nutritional inputs, but also the effect of disease, such as the acute phase response. Malavolta *et al.*[233] have developed a method based on using small monolithic AEC coupled to ICP-MS (SAX1-S, 50 × 1 mm i.d) to investigate the proteins involved with homeostasis and function of Cu, Fe, Se and Zn by the analysis of human plasma and serum. The gradient elution system used two identical columns that were switched back and forth between two flow paths, one for the separation and the second a regeneration eluent, which allowed for column washing and re-equilibrium in a timely manner. The mobile

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phase was also passed through a column containing a Chelex 100 chelating resin prior to use in an attempt to lower the trace element content. Two different calibration methods were described. The peak area normalisation method used the total concentration of the element of interest to determine the protein content by the proportion eluting as each peak. By contrast the normal calibration curve method used post-column infusion of standards and the element "mass-flow" to determine the concentration of the standards, in an analogous way to the species non-specific IDMS approach. This second method overcame the need for molecular standards, whereas the former required two measurement steps, with all the inherent disadvantages this produces. The chromatograms provided in the report identify peaks which are difficult to distinguish from the baseline signal. For Cu, Fe and Zn this is due to the retention of these elements on the column followed by slow release as the elution strength of the mobile phase increases with the gradient elution. For Se the effect seems to be due to the poor LOD for the isotope used. The clear retention of Cu, Fe and Zn on the column, followed by slow leaching off is clearly a major flaw with the column used, for which no explanation is given. Presumably these elements, which do not normally occur as ionic species in serum, have been stripped from the proteins under study making them available for acquisition by apo-proteins with vacant binding sites. Unfortunately no other chromatographic system was used to verify the reported results. The other problem with the method is that HSA, the major metal binding protein in serum, elutes as 2 peaks separated by more than 2 minutes. In all, it is unclear what the advantages of measuring the trace element binding proteins actually is compared to accurately measuring the total metal concentration. Possibly, this relates to different disease states but the authors provide no evidence of this in their report as they do not analyse samples which would reflect this. Also to be of use in a real clinical setting where hundreds of samples are measured for these elements in a single run, some indication of the robustness of the system over many more samples is required, particularly as there were problems of metal build-up and subsequent elution from the column, which were not resolved using the column clean-up wash. Measurement of the bioactive forms of vitamin B_{12} in the human diet is extremely important from a nutritional perspective because it has a vital role in important homeostatic functions such as: the formation of red blood cells; DNA synthesis; and the central nervous system. Foodstuffs of animal origin are the major source of vitamin B_{12} in the human diet. Szterk *et al.*[248] have used a small bore (2 mm i.d.) HPLC-ES-MS method with gradient elution separation, to determine the four most important bioactive forms of this vitamin; methylcobalamin, adenosine cobalamin, hydroxycobalamin and cyanocobalamin, in different forms of beef. The method LODs reported were between 3 and 80 $\mu g\ L^{-1}$ and the results showed that hydroxycobalamin was the dominant form of vitamin B_{12} in beef liver at 48% of the total B_{12} concentration, whereas adenosine cobalamin was the main form in sirloin. Cooking the meat reduced the amount of cobalamin that was measured in the samples. However, it was not clear whether this was due to the breakdown of the analyte, as proposed by the authors or due to a change in the sample matrix making the vitamin less extractable.

Metals and their associated metalloproteins are involved in a number of biochemical processes, including those associated with hypertension which is an established risk factor for stroke. The group of Caruso has been working on *the development of metallomic techniques* to investigate various forms of stroke. They have studied metal containing proteins in blood plasma using SEC-ICP-MS, followed by HPLC-ES-IT-MS using a microfluidic HPLC-chip column for peptide measurements after trypsin digestion and database searches to identify the proteins.[249] Using the total concentration of the metal associated with the metalloproteins containing that element and separated using SEC, the Mg, Mn, Cu, Se concentrations were statistically different when compared between stroke mimics (control group) *vs.* ischemic stroke patients and ischemic stroke patients *vs.* haemorrhagic stroke patients. Lead concentrations were statistically different when compared between stroke mimics *vs.* ischemic stroke patients and Mo levels were statistically the same among the three groups. Although specific metalloprotein biomarkers for stroke were not identified, the preliminary study showed scope for further investigation. In a similar vein[250] the same group have also studied the Se metallome in an effort to find biomarkers for cerebral vasospasm following subarachnoid hemorrhagic stroke. The analytical protocol once again involved SEC-ICP-MS as a first step to isolate the selenoproteins. This was followed by 1D GE, excision and in-gel trypsin digestion of the individual protein bands and analysis of the peptide fragments by MALDI-TOF-TOF-MS. Six proteins including: albumin preprotein; beta globin; carbonic anhydrase I; transthyretin precursor; peroxiredoxin I; and cystatin S precursor, were identified. Of these proteins all may be feasible as biomarkers of cerebral vasospasm, but the most promising was felt to be transthyretin precursor. Strangely no *m*/*z* corresponding to known Se species were detected in the SEC fraction containing the largest amount of Se. This illustrates one of the major problems with both approaches, in that the proteins identified in the isolated SEC fractions may not actually contain any metal(loid) or be a metalloprotein, but could just be co-eluting with a metal containing compound. The resolution obtained with SEC is not a high and one would expect a multitude of proteins to be present in the "isolated" fractions. It's also unclear what advantage focusing on the metalloproteins has in comparison with the other well established proteomics methods available which consider the complete proteome rather than just the metallome. Although the authors show some link between metals and the disease this approach could well lead investigations away from better biomarkers. A different metallomic approach[251] has been developed to investigate transgenic and non-transgenic soyabean seeds using 2D HPLC-ICP-MS and HPLC-ES-MS-MS. In this protocol herbicide resistant transgenic soyabean seeds and normal seeds were subjected to an ammonium acetate buffer (pH 7.2) extraction procedure followed by separation into three metal (Co, Cu, Fe, Mg, Mn and Zn) containing fractions by SEC. The fractions were freeze-dried, redissolved in Tris buffer (pH not reported) and separated using AEX into 7 further fractions,

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which were lyophilised and digested using trypsin, prior to analysis by HPLC-ES-MS-MS using a C_{18} column. The resulting peptides were then searched against the NCBI and Swiss-Prot databases using the MASCOT search engine in the usual proteomics approach. A total of 33 proteins were identified amongst which some related to metals were found, although the majority of the proteins do not appear to bind metals, so it is unclear what function the metals studied have in relation to them. Further work, perhaps using IMAC, to refine the isolation of the metal-binding molecules would appear necessary.

6.2 Tagging and labelling of molecules

Elemental labelling is an expanding area of research and encompasses the *indirect determination of biomolecules by labelling with metals*, nanoparticles or quantum dots. A variety of labelling methods are being investigated but all involve a ligand or antibody which has a metal attached, either by chelation or covalent bonding, that tags the target molecule. The label is usually a 'heavy' metal, lanthanide, nanoparticle or quantum dot. This approach has been applied to a variety of research areas including: the measurement of peptides, proteins and antibodies in a range of applications including biomedical science,[252] medicine[253] and art conservation.[254]

The *strategies, methods and chelators employed to label biomolecules* such as proteins, peptides and antibodies have started to be investigated and optimised. A group from NIST[255] evaluated the use of microwave energy when metal-labelling peptides for measurement by RP HPLC-ICP-MS. A microwave-assisted lanthanide labelling procedure was developed and applied to derivatise the peptides angiotensin I and II, and bradykinin, using the macrocyclic bifunctional chemical chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetic acid), which significantly improved the lanthanide labelling yield and reduced reaction times compared to bench top labelling procedures. The bio-conjugation step required buffering the samples to pH 8, and reaction of the peptides with DOTA NHS ester for 5–10 min using a temperature ramp and a hold program to a maximum of 60 °C at 300 W power. Lanthanide spikes of Ho, Tb or Tm in formic acid (0.1% m/m) at pH 6 were introduced and the samples were then heated again in the MW for 5 min to chelate the metal to the DOTA-derivatised peptides. Polymeric RF SPE cartridges (Strata X and Oasis HLB) were used to remove the unconjugated metals. Both MALDI-TOF-MS and ES-MS-MS were used to evaluate labelling efficiencies, which were approximately 55% for the microwave method, compared to 15% for the bench-top approach. The RP separation used a microbore C_{18} column, an acetonitrile gradient, which required O_2 addition and the spray chamber cooled to −5 °C to allow the plasma to tolerate the high organic modifier loading (98.5% v/v). The MSA calibration scheme was outlined in detail and applied by labelling the standard and sample with different lanthanides and combining the derivatised solutions, prior to the SPE step. Correction of the lanthanide signals for oxide formation in the ICP-MS was required to achieve accurate results. Whilst not used on real-world samples, the methodology showed promising recoveries of 99.1% for bradykinin using Tb as the spike label. Jakubowski and co-workers have compared[256] different chelates for lanthanide labelling of antibodies for application in biomedical assays utilising LA-ICP-MS detection. The lanthanide labelling of antibodies using the metal coded tagging (MeCAT) approach was investigated and the reaction conditions were optimised for application in a Western blot immunoassay analysed by LA-ICP-MS. The MeCAT labelling strategy was compared with two other commercially available labelling reagents, MAXPAR™ and SCN-DOTA. All three of these approaches involved the use of a bi-functional ligand containing a portion which strongly chelated a rare earth metal tag. This moiety was reacted with a specific amino acid group on the antibody. The SCN-DOTA covalently bonded to ε-amino acid groups of lysine residues and the DOTA macrocycle complexed the Tb lanthanide tag. The other two methods. MeCAT and MAXPAR™, both label *via* a maleimide group which binds to the sulfhydryl residues after partial reduction of the antibody's cysteine-based disulfide bridges. The MeCAT approach uses the DOTA complex in a similar manner to SCN-DOTA, whereas MAXPAR™ uses a polymer chain containing diethylenetriaminetetraacetic acid (DTTA) a linear metal chelating compound, which increases the number of Tm lanthanide tags per binding site. As a proof-of-principle experiment, chemically induced alterations of cytochrome P450 protein expression were investigated and the suitability of the differentially labelled antibodies for Western blot immunoassays of a complex liver microsomal protein fraction tested. Lower fmol range LODs were reported for the Western blot application using MeCAT and MAXPAR™ as element labelling reagents, whereas sub-fmol LODs could be achieved in a dot blot experiment for the pure antibodies.

Further work on *MeCAT labelling* has focused on the absolute quantification of proteins rather than relative quantification using analyte abundance. In a series of recent papers protocols have been reported for the application of MeCAT labelling to: the thermally induced expression of the clotting factor aprotinin by 2D GE followed by FI-ICP-MS and nanoHPLC-ES-MS-MS;[257] the measurement of lysosyme, BSA and transferrin by 2D-HPLC-ICP-MS;[258] and HSA and transferrin in human serum by 2D GE with LA-ICP-MS.[259] The Linscheid group show[257] that the MeCAT labelling process can be applied to the measurement of a single protein, in this case recombinantly produced aprotinin. This could be achieved using both a bottom–up strategy, after labelling of the peptides resulting from tryptic digest and also a top-down process, labelling the cysteine residues of the intact protein. The bottom–up method requires that characteristic peptides for the protein are identified using nanoHPLC-ESI-MS-MS and then synthesised, prior to labelling for use as internal standards for absolute quantitation. To illustrate the application of the method, peptides from before thermal induction of aprotinin were labelled with Lu and those after with Ho, and HPLC-ICP-MS was used to demonstrate the difference in level of expression. This would not be possible with relative quantification methods because the protein is not expressed at normal temperatures. The main drawback with this approach was the 7 hours chromatographic run required to separate the peptides. The top–down system, labelling the intact protein directly, has

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the advantage that information is not lost due to the digestion step and sample complexity is kept to a minimum. Using the same labelling approach, proteins were separated by 2D-GE and the stained spots excised and analysed by ICP-MS. The results showed the absence of thermally induced protein in the unheated treatment as before. Accurate quantification using this approach requires the tagged and untagged forms of a protein to migrate in electrophoresis in a similar manner. This was not elucidated in the presented results, where the MeCAT tagged species are located in a different manner to the unlabelled species. To assess the accuracy delivered by both MeCAT workflows, the results were compared to a reference method. The top–down approach gave recoveries of 174% and 198% for the two thermal comparisons and the bottom–up approach yielded 55% and 40%. The precision was reported as between 24 and 45%.

The use of *tags such as nanoparticles or quantum-dots* containing a large number of metal atoms increases the sensitivity of the method compared to other labelling techniques. A method for the quantification of human total urinary protein by using CdTe quantum dots as labels and ICP-MS detection has been developed.[260] The CdTe quantum dots were directly conjugated to the proteins and the ICP-MS signal intensity of cadmium was proportional to the concentration of protein in the urine. The linear range was given as 4 orders of magnitude and interferences from common inorganic ions and other compounds was reported to be minimal. The method was applied to the measurement of the total protein in 50 human urine samples and the results obtained were in good agreement with those using the conventional method. The advantages over simpler current methods was not established. Jarujamrus *et al.*[261] used gold nanoparticles to measure chloramphenicol (CAP) using an immunoassay and ICP-MS. Polyclonal rabbit anti-mouse immunoglobulins (anti-mouse IgG) were pre-coated on the 96-well polystyrene microplate solid support to allow the retention of mouse monoclonal to chloramphenicol (MAb-anti-CAP) antibody-CAP on the plates. Samples containing CAP as an antigen premixed with CAP–BSA protein labeled with AuNPs as an immunogenic tag were added to the MAb-anti-CAP bound solid support, physically separated from non-reacting molecules. The AuNPs were measured by ICP-MS to indirectly determine the CAP concentration in the samples. For 10 nm AuNPs, the optimal condition for CAP–BSA protein conjugation was pH 9.5 and 120 mg L^{-1} of CAP–BSA protein. The detection limit, linearity range, and precision (intra-assay, inter-assay) were 4.52 ng mL^{-1}, 0–20 ng mL^{-1}, and less than 20%, respectively.

An ingenious use of a metal-tag for the *detection of DNA single-nucleotide polymorphisms (SNPs)*, which utilises the displacement of the Hg label, rather than its presence on the determined moiety, has been reported for the quantification of SNPs.[262] In this case the Hg-tag was attached to a thymine rich single strand DNA capture sequence forming a loop, analogous to a molecular beacon, with the Hg atom bound between two interstrand thymine bases. On exposure to the target DNA the probe unfurled to hybridise with the DNA sequence, releasing the Hg-tag which is detected by CE-ICP-MS. The CE step effectively separated the released Hg-tag from the remaining Hg-labelled capture DNA sequence. As SNPs are introduced into the measured single strand DNA, less of the Hg is released from the capture loop. As this occurs stoichiometrically it is possible to measure the number and magnitude of SNPs present by the concentration of unbound Hg. The method can only identify the specific SNP by the capture DNA sequence used, which makes the approach cumbersome and somewhat tedious as the capture probe would have to be specific to the SNP being investigated. However, this is also the case for molecular beacon probes, upon which this work is based. The methodology was only employed with synthetic DNA sequences and not to real world situations, so it is difficult to see how the approach, whilst novel, improves upon the current methods for SNP detection and quantitation.

6.3 Imaging by LA-ICP-MS

The use of LA-ICP-MS for imaging is an expanding area of research, particularly in the biomedical field. It involves the use of direct laser sampling to provide a spatial analysis that can be represented as an elemental map or image of a material, or a planar separation. The field also encompasses direct measurement of hetero or metal containing molecules and indirect determination by labelling molecules with metals or nanoparticles.

Two recent *reviews highlight the quantification strategies that are essential to obtaining useful data from LA-ICP-MS* studies. Hare *et al.*[263] cover a range of calibration approaches that have been used to assess the spatial distribution of specific trace elements in a range of human and animal derived biomaterials, including: hair, thin brain tissue sections, human teeth, animal liver sections, leaves, and other materials such as glass, geological materials and PAGE gels. Internal standardisation, discussed in some detail in this review, is necessary with LA-ICP-MS analysis to overcome changes in elemental response. These effects may be caused by fluctuations in laser output power, variations in the mass and transport efficiency of ablated material, plasma instability and surface imperfections (particularly with gels) leading to slight changes in height relative to the focus position of the laser. The most suitable approach is to use a naturally occurring element found within the matrix of the material under investigation. Ideally the element used should be present at a known concentration and have similar ablation properties as the analyte. Some examples presented include: Ca for signal normalisation in teeth, S in hair analysis, and ^{13}C in tissue samples. Alternatives to the use of elemental internal standardisation as a means to compensate for variation in ablated mass have been proposed including normalisation by acoustic wave generation and measurement of light scattered by ablated particles. The calibration approaches discussed included: matrix matched standards and pelleted CRMs; on-line addition of solution standards; IDMS after spiking or doping of different materials; and direct printing of standards onto a suitable support. Whilst this review dealt predominantly with total and spatial elemental analysis it did also include a discussion of the analysis of 2D PAGE gels, using the hetero-atoms such as S present in the separated protein spots and the

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metals also present, to quantify the amount of protein. The calibration and internal standardisation methods discussed were shown to be applicable to the determination of metalloproteins using LA-ICP-MS. However, the second review[264] specifically addressed the measurement of heteroatom containing proteins and proteins labelled with metal-containing tags. The main and most useful feature of LA-ICP-MS is its spatial resolution (<1 μm), which is reported to be better than micro-XRF. However, the downside of this is the length of time required for data acquisition, which can be anything up to 30 h for a high resolution "image" of a 5 mm^2 specimen. The lack of validated quantification strategies is another limitation of the approach. The bioimaging of elements in human and animal tissues; tissue sample preparation; quantification and internal standardisation; analysis of heteroatom-containing proteins and electrophoresis gels; and elemental tagging for peptide and protein analysis were also assessed in the review. Whilst biomedical applications of the technique are stressed in the paper, it is difficult to see how it could be applied in mainstream biomedical science. Transfer out of the research laboratory into the clinic does not seem likely at the current level of development.

One area of biomedical science that would benefit from the development of LA-ICP-MS applications is histopathology, which currently uses staining and immunochemistry of tissue biopsy samples to identify biomarkers of disease.

The group of Jakubowski has worked on the use of immunochemical labelling and ICP-MS for a number of years and have now developed a *multiplexed immunohistochemical method for the detection of tumour biomarkers* in breast cancer tissue using LA-ICP-MS.[265] This approach is essentially based on the biopsy samples and protocols widely in use in histopathological centres. Three antibodies with a high degree of selectivity for a specific tumour marker were labelled, each with a different lanthanide. Antihuman epidermal growth factor receptor 2 (Her 2), anticytokeratin 7 (CK 7) and antimucin 1 (MUC 1) were labelled with Ho, Tm and Tb respectively using a DOTA complex as a linker. Conventional formalin-fixed paraffin-embedded tissue blocks of human breast cancer biopsies were sectioned (5 μm thick) by microtome and then incubated with the labelled antibodies. The sections were then mounted onto microscope glass slides and analysed by LA coupled to SF-ICP-MS. The instrumental parameters were optimised for optimal signal-to-background ratio and the total analysis time for a tissue (6 mm × 5 mm) was 1 h using a 200 μm laser spot size and a 200 μm s^{-1} scan speed. Whilst inferior in spatial resolution to light microscopy, by a factor of 10 to 100, the advantages of the approach are: the possibility for quantification; simultaneous detection of several biomarkers within one tissue section even if they are colocalised; and shorter analysis time than conventional histopathological methods due to multiplexing several markers simultaneously.

The *analysis of macromolecules separated using gel-electrophoresis or microarrays using LA-ICP-MS* has been the subject of a number of reports. A method was developed for the determination of ovalbumin by labelling with *p*-hydroxy-mercuribenzoic acid (*p*HMB), separation by PAGE and measurement of Hg by LA-ICP-MS.[266] The ablation process was optimised in terms of laser repetition rate (20 Hz), spot size (160 μm) and scan velocity (50 μm s^{-1}). Two different quantification strategies were studied: external calibration using standards of the derivatised protein and ^{13}C correction; and label-specific IDA using pHMB enriched in the isotope ^{199}Hg. Normalisation to the matrix element C significantly improved the calibration correlation and precision. The IDA approach overcame the inhomogeneous distribution of the protein within the gel band, to provide accurate results and could also compensate for analyte loss and degradation as soon as complete mixture of spike and sample was achieved. The estimated LOD was 160 fmol for ovalbumin. In contrast to earlier studies using metals naturally present in proteins, no loss of mercury was observed during separation under denaturing conditions and other sample preparation steps. Using label-specific IDA, the measured isotope ratios in the gel corresponded to recoveries between 95% and 103%. The determination of Pt-containing protein complexes in biological samples,[267] after cisplatin treatment, has been developed using GE and spatial analysis by LA-ICP-SF-MS. Spots containing Pt were excised and in-gel digested with trypsin so the peptides produced could be identified using nHPLC-ES-LTQ-FT-MS/MS. The influence of protein separation conditions, staining and gel processing prior to LA on Pt-protein bonds preservation were evaluated using standard proteins incubated with cisplatin. Separation under non-reducing conditions followed by either Coomassie blue brilliant or silver staining were both successful, achieving a suitable resolution between protein bands. Direct LA-ICP-MS analysis of glycerol-treated dried gels for Pt-protein monitoring resulted in better sensitivity, more reliable relative Pt signals and a simpler and less time-consuming approach compared to the analysis of blotted membranes. Ablation of the gels identified the Pt containing spots and the colocalised proteins could then be identified by molecular MS, making it unnecessary to run several gels in parallel for separate Pt detection and protein identification. However, the need to digest the proteins for characterisation means that information on cisplatin–protein binding is lost, which would appear to be a major drawback of the approach. The measurement of holoceruloplasmin (hCP) in serum, using LA-ICP-MS to interrogate a microarray chip has been reported.[268] The PDMS microarray chips with 150 μm diameter pillars were manufactured and the surface of each pillar was reacted with 3-glycidoxypropyl-trimethylsilane as a linker to immobilise IgG for use as an antibody to capture hCP. Human serum (126 pL) was loaded on each pillar of the microarray chip and analysed by LA-ICP-MS in single-shot mode. The determined concentration of hCP was compared with that of the total ceruloplasmin estimated by competitive ELISA, from which the concentration of apoceruloplasmin was determined. The main application of this technology was reported to be in the diagnosis of Wilsons and Menkes disease, which are both extremely rare inherited disorders of copper metabolism affecting 1 : 100 000 of the UK population. Unfortunately it is difficult to see how this method could be specific enough using only IgG as the capture molecule to improve on the current immunochemical methods used. Whilst the manufacture of the microarray chip (not to be confused with a DNA microarray) is

novel, the methodology was not validated against a control method currently in use to diagnose these rare diseases.

Abbreviations

2D	two dimensional
AAS	atomic absorption spectrometry
AB	arsenobetaine
AC	arsenocholine
AEC	anion exchange chromatography
AED	atomic emission detection
AFS	atomic fluorescence spectrometry
ALS	amyotrophic lateral sclerosis
AMS	accelerator mass spectrometry
APDC	ammonium pyrrolidine dithiocarbamate
ATP	adenosine triphosphate
BC	Before Christ
BCR	Community Bureau of Reference
BH	borohydride
BMI	body mass index
BSA	bovine serum albumin
CAP	chloramphenicol
CCD	charge coupled detector
CE	capillary electrophoresis
CTAB	cetyl trimethylammonium bromide
CMC	carboxymethyl cellulose
CRM	certified reference material
CT	cryo trapping
CV	cold vapour
DBT	dibutyltin
DGT	diffusive gradients in thin films
DIT	3,5-diiodo-tyrosine
DLLME	dispersive liquid–liquid microextraction
DM	dry matter
DMA	dimethylarsenic
DMAAG	dimethylarsonio-adenosine glutathione
DMAE	dimethylarsinoylethanol
DMDSe	dimethyldiselenide
DMSe	dimethylselenide
DMTA	dimethylthioarsinic acid
DNA	deoxyribonucleic acid
DOTA NHS	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DPC	1,5-diphenylcarbazide
DPhT	diphenyltin
EC	electrochemistry
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ES	electrospray
ESI-LTQ-FT-MS/MS	electrospray linear ion trap tandem mass spectrometry
ES-MS	electrospray mass spectrometry
ETAAS	electrothermal atomic absorption spectrometry
EtHg	ethylmercury
EXAFS	extended X-ray absorption fine structure
FAAS	flame atomic absorption spectrometry
FI-AFS	flow injection atomic fluorescence
FI-ICP-MS	flow injection inductively coupled plasma mass spectrometry
FTIR	fourier transform infrared
GC	gas chromatography
GC-AED	gas chromatography atomic emission spectrometry
GC-MS	gas chromatography mass spectrometry
Gd-BOPTA	gadobenate
Gd-BT-DO3A	gadobutrol
Gd-DOTA	gadoterate
Gd-DTPA	gadopentetate
GD-DTPA-BMA	gadodiamide
GE	gas electrophoresis
GF-AAS	graphite furnace atomic absorption spectrometry
GPC	gel permeation chromatography
GPx	glutathione peroxidase
hCP	holoceruloplasmin
HG	hydride generation
HG-QF-AAS	hydride generation quartz furnace atomic fluorescence spectrometry
HILIC	hydrophilic interaction liquid
HPLC	high performance liquid chromatography
HPLC-ES-IT-MS	high performance liquid chromatography electrospray ion trap mass spectrometry
HPLC-ICP-MS	high performance liquid chromatography inductively coupled plasma mass spectrometry
HY	hybrid
IAEA	International Atomic Energy Agency
iAs	inorganic arsenic
IC	ion chromatography
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
ID	isotope dilution
IDA	isotope dilution analysis
IDMS	isotope dilution mass spectrometry
IgG	immuno gamma globulin
iHg	inorganic mercury
IMAC	immobilised metal affinity chromatography
iSb	inorganic antimony
LA	laser ablation
LA-ICP-MS	laser ablation inductively coupled plasma mass spectrometry
LC	liquid chromatography
LC-ICP-MS	liquid chromatography inductively coupled plasma mass spectrometry
LLE	liquid–liquid extraction
LLME	liquid–liquid–liquid microextraction
LOD	limit of detection
μR-XRF	micro-synchrotron radiation X-ray fluorescence
μ-XAFS	micro-X-ray absorption fine structure spectrometry
μXANES	micro-X-ray absorption near-edge structure
μ-XAS	micro-X-ray absorption spectroscopy
μ-XRD	micro-X-ray diffraction
μ-XRF	micro-X-ray fluorescence
MAE	microwave assisted extraction

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MALDI	matrix-assisted laser desorption ionization
MC-ICP-MS	multicollector inductively coupled plasma mass spectrometry
MeCAT	metal coded tagging
MeHg	methyl mercury
MeOH	methanol
MeSeCys	methylselenocysteine
MIP-OES	microwave induced plasma optical emission spectrometry
MIT	3-iodo-tyrosine
MMA	monomethylarsenic
MPhT	monophenyltin
MRI	magnetic resonance imaging
MS	mass spectrometry
MSA	methanesulphonic acid
MT	metallothioniens
$NaPr_4B$	sodium tetrapropyl borate
$NeBEt_4$	sodium tetraethyl borate
NIES	National Institute for Environmental Studies
NIST	National Institute of Standards and Technology
NMIJ	National Measurement Institute of Japan
NP	nanoparticle
OPA	Opalinus Clay
OTC	organotin compounds
PAG	poly-aluminium granulates
PAGE	polyacrylamide gel electrophoresis
PBET	Physiologically Based Extraction Test
PCA	principal component analysis
PDMS	polydimethylsiloxane
PEG	polyethylene glycol
*p*HMB	*p*-hydroxy-mercuribenzoic acid
PIXE	particle-induced X-ray emission
PTFE	poly(tetrafluoroethylene)
PVDF	polyvinylidene difluoride
RP	reversed phase
RT	room temperature
SAX	strong anion exchange
SBAE	strong base anion exchange
SBTD	*N*,*N*′-bis-(alpha-methylsalicylidene)-2, 2-dimethyl-1,3-propanediimine
SCX	strong cation exchange
SDS	sodium dodecylsulfate
SDS-PAGE	sodium dodecylsulfate polyacrylamide gel electrophoresis
SeAlb	selenoalbumin
SEC	size exclusion chromatography
$SeCyst_2$	selenocystine
SelP	selenoprotein
SEM	scanning electron microscopy
SeMeSeCyst	selenomethylselenocystine
SeMet	selenomethionein
SF-ICP-MS	sector field inductively coupled plasma mass spectrometry
SHIME	simulator of the Human Intestinal Microbial Ecosystem
SNPs	single-nucleotide polymorphisms
SPE	solid phase extraction
SPM	suspended particulate matter
SPME	solid phase micro-extraction
SRM	solid phase microextraction
SR-XRF	synchrotron radiation X-ray fluorescence
ssID-ICP-MS	species specific isotope dilution inductively coupled plasma mass spectrometry
SSIDMS	species specific isotope dilution mass spectrometry
STXM	scanning transmission X-ray microspectroscopy
SUIDMS	species unspecific isotope dilution mass spectrometry
TBT	tributyltin
TEL	tetraethyllead
TGA	thermogravimetric analysis
TMAH	tetramethylammonium hydroxide
TMAO	trimethylarsine oxide
TMSb	trimethylantimony
TMSe	trimethylselenium
TOF	time-of-flight
UAE	ultrasound-assisted extraction
UV	ultraviolet
XANES	X-ray absorption near-edge structure
XAS	X-ray absorption spectroscopy
XRD	X-ray diffraction
XRF	X-ray fluorescence.

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References

1 O. T. Butler, W. R. L. Cairns, J. M. Cook and C. M. Davidson, *J. Anal. At. Spectrom.*, 2012, **27**(2), 187–221.
2 A. Taylor, M. P. Day, J. Marshall, M. Patriarca and M. White, *J. Anal. At. Spectrom.*, 2012, **27**(4), 537–576.
3 E. H. Evans, C. D. Palmer and C. M. M. Smith, *J. Anal. At. Spectrom.*, 2012, **27**(6), 909–927.
4 M. West, A. T. Ellis, P. J. Potts, C. Streli, C. Vanhoof, D. Wegrzynek and P. Wobrauschek, *J. Anal. At. Spectrom.*, 2012, **27**(10), 1603–1644.
5 S. Carter, A. S. Fisher, M. W. Hinds and S. Lancaster, *J. Anal. At. Spectrom.*, 2012, **27**(12), 2003–2053.
6 D. Beauchemin, D. E. Matthews, *The Encyclopedia of Mass Spectrometry, Vol. 5*, Elsevier Science & Technology, Amsterdam, 2013.
7 K. G. Heumann, in *Isotopic Analysis: Fundamentals and Applications Using ICP-MS*, ed. F. V. a. P. Degryse, Wiley-VCH, Weinheim, 2012, pp. 189–233.
8 J. I. G. Alonso, M. Moldovan and P. R. González, *Isotope Dilution Mass Spectrometry*, Royal Society of Chemistry, Cambridge, 2013.
9 M. S. Bank, *Mercury in the Environment: Pattern and Process*, University of California Press, Los Angeles, 2012.
10 S. G. Capar, P. Szefer, *Methods of Analysis of Food Components and Additives, Second Edition*, ed. S. Otles, CRC Press, Boca Raton, 2011.
11 A. A. Meharg, F.-J. Zhao, *Arsenic & Rice*, Springer, Dordrecht, 2012.
12 M. Asensio-Ramos, L. M. Ravelo-Perez, M. A. Gonzalez-Curbelo and J. Hernandez-Borges, *J. Chromatogr., A*, 2011, **1218**(42), 7415–7437.

Published on 02 July 2013. Downloaded by 500095 on 06/08/2013 11:57:27.

13 D. Han and K. H. Row, *Microchim. Acta*, 2012, **176**(1–2), 1–22.
14 D. Das, U. Gupta and A. K. Das, *TrAC, Trends Anal. Chem.*, 2012, **38**, 163–171.
15 C. H. Latorre, J. A. Mendez, J. B. Garcia, S. G. Martin and R. M. P. Crecente, *Anal. Chim. Acta*, 2012, **749**, 16–35.
16 A. V. Herrera-Herrera, M. A. Gonzalez-Curbelo, J. Hernandez-Borges and M. A. Rodriguez-Delgado, *Anal. Chim. Acta*, 2012, **734**, 1–30.
17 K. Pyrzynska, *TrAC, Trends Anal. Chem.*, 2012, **32**, 100–112.
18 K. Pyrzynska, *Int. J. Environ. Anal. Chem.*, 2012, **92**(11), 1298–1311.
19 C. Bendicho, I. De La Calle, F. Pena, M. Costas, N. Cabaleiro and I. Lavilla, *TrAC, Trends Anal. Chem.*, 2012, **31**, 50–60.
20 R. P. Li, Y. L. Guo and Q. Yuan, *J. Liq. Chromatogr. Relat. Technol.*, 2011, **34**(13), 1112–1132.
21 S. R. Haider, B. L. Sharp and H. J. Reid, *TrAC, Trends Anal. Chem.*, 2011, **30**(11), 1793–1808.
22 A. Sussulini and J. S. Becker, *Metallomics*, 2011, **3**(12), 1271–1279.
23 M. Ryvolova, V. Adam and R. Kizek, *J. Chromatogr., A*, 2012, **1226**, 31–42.
24 P. Kuban and A. R. Timerbaev, *Electrophoresis*, 2012, **33**(1), 196–210.
25 Y. L. Yu, Y. Jiang, M. L. Chen and J. H. Wang, *TrAC, Trends Anal. Chem.*, 2011, **30**(10), 1649–1658.
26 P. Rodriguez-Gonzalez, V. N. Epov, C. Pecheyran, D. Amouroux and O. F. X. Donard, *Mass Spectrom. Rev.*, 2012, **31**(4), 504–521.
27 D. Profrock and A. Prange, *Appl. Spectrosc.*, 2012, **66**(8), 843–868.
28 B. Meermann and M. Sperling, *Anal. Bioanal. Chem.*, 2012, **403**(6), 1501–1522.
29 F. van Heuveln, H. Meijering and J. Wieling, *Bioanalysis*, 2012, **4**(15), 1933–1965.
30 Z. Long, Y. M. Luo, C. B. Zheng, P. C. Deng and X. D. Hou, *Appl. Spectrosc. Rev.*, 2012, **47**(5), 382–413.
31 Z. Long, C. Chen, X. D. Hou and C. B. Zheng, *Appl. Spectrosc. Rev.*, 2012, **47**(7), 495–517.
32 M. Welna, A. Szymczycha-Madeja, E. Stelmach and P. Pohl, *Crit. Rev. Anal. Chem.*, 2012, **42**(4), 349–365.
33 R. B. Khouzam, J. Szpunar, M. Holeman and R. Lobinski, *Pure Appl. Chem.*, 2012, **84**(2), 169–179.
34 L. Ruzik, *Talanta*, 2012, **93**, 18–31.
35 A. M. Carey, E. Lombi, E. Donner, M. D. de Jonge, T. Punshon, B. P. Jackson, M. L. Guerinot, A. H. Price and A. A. Meharg, *Anal. Bioanal. Chem.*, 2012, **402**(10), 3275–3286.
36 F. Drewnick, *Anal. Bioanal. Chem.*, 2012, **404**(8), 2127–2131.
37 I. Fulara and M. Czaplicka, *J. Sep. Sci.*, 2012, **35**(16), 2075–2087.
38 M. Cotte and J. Susini, *Actual. Chim.*, 2011, (356–57), 113–115.
39 D. Olivares, M. Bravo, J. Feldmann, A. Raab, A. Neaman and W. Quiroz, *J. AOAC Int.*, 2012, **95**(4), 1176–1182.
40 R. E. Wolf, S. A. Morman, P. L. Hageman, T. M. Hoefen and G. S. Plumlee, *Anal. Bioanal. Chem.*, 2011, **401**(9), 2733–2745.
41 M. D. Joshi and J. L. Anderson, *RSC Adv.*, 2012, **2**(13), 5470–5484.
42 Y. L. Zhang and S. B. Adeloju, *Anal. Chim. Acta*, 2012, **721**, 22–27.
43 A. Jain and K. K. Verma, *Anal. Chim. Acta*, 2011, **706**(1), 37–65.
44 L. Kocurova, I. S. Balogh, J. Sandrejova and V. Andruch, *Microchem. J.*, 2012, **102**, 11–17.
45 K. Xie, K. Huang, L. R. Yang, P. H. Yu and H. Z. Liu, *Ind. Eng. Chem. Res.*, 2011, **50**(22), 12767–12773.
46 G. A. Zachariadis and E. Rosenberg, *J. Sep. Sci.*, 2012, **35**(9), 1132–1137.
47 S. Yalcin, H. Filik and R. Apak, *J. Anal. Chem.*, 2012, **67**(1), 47–55.
48 I. Hagarova, M. Bujdos, P. Matus and L. Canecka, *Chem. Listy*, 2012, **106**(2), 136–142.
49 I. Hagarova, P. Matus, M. Bujdos and J. Kubova, *Acta Chim. Slov.*, 2012, **59**(1), 102–108.
50 S. Bahar and R. Zakerian, *J. Braz. Chem. Soc.*, 2012, **23**(5), 944–950.
51 G. A. Zachariadis and E. Trikas, *Int. J. Environ. Anal. Chem.*, 2012, **92**(4), 375–381.
52 Y. W. Wu, J. Zhang, J. F. Liu, L. Chen, Z. L. Deng, M. X. Han, X. S. Wei, A. M. Yu and H. L. Zhang, *Appl. Surf. Sci.*, 2012, **258**(18), 6772–6776.
53 X. P. Yao, Z. J. Fu, Y. G. Zhao, L. Wang, L. Y. Fang and H. Y. Shen, *Talanta*, 2012, **97**, 124–130.
54 X. X. Zhang, S. S. Tang, M. L. Chen and J. H. Wang, *J. Anal. At. Spectrom.*, 2012, **27**(3), 466–472.
55 B. W. Yang and Z. F. Fan, *J. Anal. Chem.*, 2012, **67**(5), 443–447.
56 L. Zhang, Z. H. Li, X. H. Du, R. J. Li and X. J. Chang, *Spectrochim. Acta, Part A*, 2012, **86**, 443–448.
57 I. Lopez-Garcia, R. E. Rivas and M. Hernandez-Cordoba, *Talanta*, 2011, **86**, 52–57.
58 A. Spolaor, P. Vallelonga, J. Gabrieli, G. Cozzi, C. Boutron and C. Barbante, *J. Anal. At. Spectrom.*, 2012, **27**(2), 310–317.
59 A. Erdem, T. Shahwan, A. Cagir and A. E. Eroglu, *Chem. Eng. J.*, 2011, **174**(1), 76–85.
60 L. Alcalde-Isorna, M. C. Barciela-Alonso and P. Bermejo-Barrera, *At. Spectrosc.*, 2011, **32**(6), 234–239.
61 I. Dakova, T. Yordanova and I. Karadjova, *J. Hazard. Mater.*, 2012, **231**, 49–56.
62 X. Y. Jia, D. R. Gong, Y. Han, T. C. Duan and H. T. Chen, *Anal. Methods*, 2012, **4**(2), 575–580.
63 C. Valls-Cantenys, M. Iglesias, J. L. Todoli and V. Salvado, *J. Chromatogr., A*, 2012, **1231**, 16–21.
64 J. L. Guzman-Mar, L. Hinojosa-Reyes, A. M. Serra, A. Hernandez-Ramirez and V. Cerda, *Anal. Chim. Acta*, 2011, **708**(1–2), 11–18.
65 S. Prikler, D. Pick and J. W. Einax, *Anal. Bioanal. Chem.*, 2012, **403**(4), 1109–1116.
66 G. Li, D. Wu, W. Y. Xie, X. M. Zhang and B. Z. Liu, *Talanta*, 2012, **95**, 36–41.

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67 J. Sun, B. He, Q. Liu, T. Ruan and G. B. Jiang, *Talanta*, 2012, **93**, 239–244.
68 J. Sun, B. He, Y. G. Yin, L. Li and G. B. Jiang, *Anal. Methods*, 2010, **2**(12), 2025–2031.
69 V. Angeli, S. Biagi, S. Ghimenti, M. Onor, A. D'Ulivo and E. Bramanti, *Spectrochim. Acta, Part B*, 2011, **66**(11–12), 799–804.
70 Z. H. Wang, Y. G. Yin, B. He, J. B. Shi, J. F. Liu and G. B. Jiang, *J. Anal. At. Spectrom.*, 2010, **25**(6), 810–814.
71 Y. G. Yin, Y. Liu, J. F. Liu, B. He and G. B. Jiang, *Anal. Methods*, 2012, **4**(4), 1122–1125.
72 J. Naozuka and C. S. Nomura, *J. Anal. At. Spectrom.*, 2011, **26**(11), 2257–2262.
73 S. D. Fernandez, N. Sugishama, J. R. Encinar and A. Sanz-Medel, *Anal. Chem.*, 2012, **84**(14), 5851–5857.
74 F. Bardelli, G. Barone, V. Crupi, F. Longo, G. Maisano, D. Majolino, P. Mazzoleni and V. Venuti, *J. Synchrotron Radiat.*, 2012, **19**, 782–788.
75 M. O. Figueiredo, T. P. Silva and J. P. Veiga, *J. Electron Spectrosc. Relat. Phenom.*, 2012, **185**(3–4), 97–102.
76 S. Cagno, G. Nuyts, S. Bugani, K. De Vis, O. Schalm, J. Caen, L. Helfen, M. Cotte, P. Reischig and K. Janssens, *J. Anal. At. Spectrom.*, 2011, **26**(12), 2442–2451.
77 E. Curti, D. Grolimund and C. N. Borca, *Appl. Geochem.*, 2012, **27**(1), 56–63.
78 C. Degueldre, M. Martin, G. Kuri, D. Grolimund and C. Borca, *J. Nucl. Mater.*, 2011, **416**(1–2), 142–150.
79 D. R. Frohlich, S. Amayri, J. Drebert, D. Grolimund, J. Huth, U. Kaplan, J. Krause and T. Reich, *Anal. Bioanal. Chem.*, 2012, **404**(8), 2151–2162.
80 A. Funatsuki, M. Takaoka, K. Oshita and N. Takeda, *Anal. Sci.*, 2012, **28**(5), 481–490.
81 M. M. Shafer, B. M. Toner, J. T. Oyerdier, J. J. Schauer, S. C. Fakra, S. H. Hu, J. D. Herner and A. Ayala, *Environ. Sci. Technol.*, 2012, **46**(1), 189–195.
82 J. A. Breier, B. M. Toner, S. C. Fakra, M. A. Marcus, S. N. White, A. M. Thurnherr and C. R. German, *Geochim. Cosmochim. Acta*, 2012, **88**, 216–236.
83 E. Koudouna, G. Veronesi, Patel, II, M. Cotte, C. Knupp, F. L. Martin and A. J. Quantock, *Biophys. J.*, 2012, **103**(2), 357–364.
84 S. Huguet, V. Bert, A. Laboudigue, V. Barthes, M. P. Isaure, I. Llorens, H. Schat and G. Sarret, *Environ. Exp. Bot.*, 2012, **82**, 54–65.
85 E. Harada, A. Hokura, I. Nakai, Y. Terada, K. Baba, K. Yazaki, M. Shiono, N. Mizuno and T. Mizuno, *Metallomics*, 2011, **3**(12), 1340–1346.
86 B. B. Chu, L. Q. Luo, T. Xu, J. Yuan, J. L. Sun, Y. Zeng, Y. H. Ma and S. Yi, *Spectrosc. Spectral Anal.*, 2012, **32**(7), 1975–1978.
87 E. Donner, C. G. Ryan, D. L. Howard, B. Zarcinas, K. G. Scheckel, S. P. McGrath, M. D. de Jonge, D. Paterson, R. Naidu and E. Lombi, *Environ. Pollut.*, 2012, **166**, 57–64.
88 S. Wonglee, T. Tada, H. Fukuda, J. Hasegawa and Y. Oguri, *Nucl. Instrum. Methods Phys. Res., Sect. B*, 2011, **269**(24), 3111–3114.
89 J. Laurette, C. Larue, C. Mariet, F. Brisset, H. Khodja, J. Bourguignon and M. Carriere, *Environ. Exp. Bot.*, 2012, **77**, 96–107.
90 Y. Morishita, K. Yamashita, T. Yoshikawa, Y. Terada, H. Nabeshi, Y. Yoshioka, N. Itoh and Y. Tsutsumi, *Pharmazie*, 2011, **66**(10), 808–809.
91 T. Narukawa, K. Inagaki, Y. B. Zhu, T. Kuroiwa, I. Narushima, K. Chiba and A. Hioki, *Anal. Bioanal. Chem.*, 2012, **402**(4), 1713–1722.
92 T. Narukawa, K. Inagaki, Y. B. Zhu, T. Kuroiwa, I. Narushima, K. Chiba and A. Hioki, *Anal. Bioanal. Chem.*, 2012, **403**(10), 2791–2793.
93 T. Zuliani, R. Milacic and J. Scancar, *Anal. Bioanal. Chem.*, 2012, **403**(3), 857–865.
94 P. Jitaru, C. Oster, G. Labarraque, S. Vaslin-Reimann and P. Fisicaro, *Accredit. Qual. Assur.*, 2011, **16**(11), 545–552.
95 C. Hansen, E. W. Hansen, H. R. Hansen, B. Gammelgaard and S. Sturup, *Biol. Trace Elem. Res.*, 2011, **144**(1–3), 234–243.
96 G. Cornelis, T. Van Gerven and C. Vandecasteele, *Waste Manage.*, 2012, **32**(2), 278–286.
97 H. M. Anawar, *Talanta*, 2012, **88**, 30–42.
98 H. Erdogan, O. Yalcinkaya and A. R. Turker, *Desalination*, 2011, **280**(1–3), 391–396.
99 N. Ben Issa, V. N. Rajakovic-Ognjanovic, A. D. Marinkovic and L. V. Rajakovic, *Anal. Chim. Acta*, 2011, **706**(1), 191–198.
100 H. Matusiewicz and M. Slachcinski, *Microchem. J.*, 2012, **102**, 61–67.
101 J. Mertens, J. Rose, R. Kagi, P. Chaurand, M. Plotze, B. Wehrli and G. Furrer, *Environ. Sci. Technol.*, 2012, **46**(13), 7310–7317.
102 M. I. An, X. X. Zhang, T. Yang, M. L. Chen and J. H. Wang, *Chin. J. Chem.*, 2012, **30**(9), 2225–2231.
103 H. Osterlund, M. Faarinen, J. Ingri and D. C. Baxter, *Environ. Chem.*, 2012, **9**(1), 55–62.
104 L. L. Chaparro, L. Ferrer, V. Cerda and L. O. Leal, *Anal. Bioanal. Chem.*, 2012, **404**(5), 1589–1595.
105 A. S. Lewis, K. R. Reid, M. C. Pollock and S. L. Campleman, *J. Air Waste Manage. Assoc.*, 2012, **62**(1), 2–17.
106 D. Sanchez-Rodas, A. S. de la Campa, V. Oliveira and J. de la Rosa, *J. Inorg. Biochem.*, 2012, **108**, 112–114.
107 M. M. Gheisari, S. M. Talebi and M. Saber-Tehrani, *Asian J. Chem.*, 2012, **24**(5), 2253–2256.
108 G. S. Yang, L. L. Ma, D. D. Xu, J. Li, T. T. He, L. Y. Liu, H. L. Jia, Y. B. Zhang, Y. Chen and Z. F. Chai, *Chemosphere*, 2012, **87**(8), 845–850.
109 M. C. Corriveau, H. E. Jamieson, M. B. Parsons, J. L. Campbell and A. Lanzirotti, *Appl. Geochem.*, 2011, **26**(9–10), 1639–1648.
110 J. A. Baig, T. G. Kazi, A. Q. Shah, G. A. Kandhro, H. I. Afridi, S. Khan, N. F. Kolachi and S. K. Wadhwa, *Int. J. Environ. Anal. Chem.*, 2012, **92**(1), 28–42.
111 R. Gardner, J. Hamadani, M. Grander, F. Tofail, B. Nermell, B. Palm, M. Kippler and M. Vahter, *Am. J. Public Health*, 2011, **101**, S333–S338.
112 G. Caumette, I. Koch, E. Estrada and K. J. Reimer, *Environ. Sci. Technol.*, 2011, **45**(23), 9917–9923.

Published on 02 July 2013. Downloaded by 500095 on 06/08/2013 11:57:27.

113 G. Caumette, I. Koch, M. Moriarty and K. J. Reimer, *Sci. Total Environ.*, 2012, **432**, 243–250.
114 A. P. Hitchcock, M. Obst, J. Wang, Y. S. Lu and T. Tyliszczak, *Environ. Sci. Technol.*, 2012, **46**(5), 2821–2829.
115 M. A. Garcia-Sevillano, M. Gonzalez-Fernandez, R. Jara-Biedma, T. Garcia-Barrera, A. Vioque-Fernandez, J. Lopez-Barea, C. Pueyo and J. L. Gomez-Ariza, *Chem. Pap.*, 2012, **66**(10), 914–924.
116 E. A. Garcia-Montalvo, O. L. Valenzuela, L. C. Sanchez-Pena, A. Albores and L. M. Del Razo, *Toxicol. Mech. Methods*, 2011, **21**(9), 649–655.
117 H. J. Wu, M. Krishnamohan, P. K. S. Lam and J. C. Ng, *Kaohsiung J. Med. Sci.*, 2011, **27**(9), 417–423.
118 C. Bergqvist and M. Greger, *Appl. Geochem.*, 2012, **27**(3), 615–622.
119 R. Larios, R. Fernandez-Martinez, I. LeHecho and I. Rucandio, *Sci. Total Environ.*, 2012, **414**, 600–607.
120 H. Castillo-Michel, J. A. Hernandez-Viezcas, A. Servin, J. R. Peralta-Videa and J. L. Gardea-Torresdey, *Appl. Spectrosc.*, 2012, **66**(6), 719–727.
121 H. S. Ferreira, M. N. Matos-Reyes, M. L. Cervera, S. L. C. Ferreira and M. de la Guardia, *Food Analytical Methods*, 2011, **4**(4), 447–452.
122 P. M. Kopittke, M. D. de Jonge, N. W. Menzies, P. Wang, E. Donner, B. A. McKenna, D. Paterson, D. L. Howard and E. Lombi, *Plant Physiol.*, 2012, **159**(3), 1149–1158.
123 A. Leufroy, L. Noel, D. Beauchemin and T. Guerin, *Anal. Bioanal. Chem.*, 2012, **402**(9), 2849–2859.
124 W. H. Li and Y. H. Liu, *Chin. J. Anal. Chem.*, 2011, **39**(12), 1912–1916.
125 A. H. Petursdottir, H. Gunnlaudsdottir, H. Jorundsdottir, A. Raab, E. M. Krupp and J. Feldmann, *Pure Appl. Chem.*, 2012, **84**(2), 191–202.
126 J. Moreda-Pineiro, E. Alonso-Rodriguez, V. Romaris-Hortas, A. Moreda-Pineiro, P. Lopez-Mahia, S. Muniategui-Lorenzo, D. Prada-Rodriguez and P. Bermejo-Barrera, *Food Chem.*, 2012, **130**(3), 552–560.
127 S. Garcia-Salgado, G. Raber, R. Raml, C. Magnes and K. A. Francesconi, *Environ. Chem.*, 2012, **9**(1), 63–66.
128 M. J. Ruiz-Chancho, M. S. Taleshi, W. Goessler and K. A. Francesconi, *J. Anal. At. Spectrom.*, 2012, **27**(3), 501–504.
129 C. Garcia-Sartal, S. Taebunpakul, E. Stokes, M. D. Barciela-Alonso, P. Bermejo-Barrera and H. Goenaga-Infante, *Anal. Bioanal. Chem.*, 2012, **402**(10), 3359–3369.
130 T. Llorente-Mirandes, M. J. Ruiz-Chancho, M. Barbero, R. Rubio and J. F. Lopez-Sanchez, *J. Agric. Food Chem.*, 2011, **59**(24), 12963–12968.
131 S. Garcia-Salgado, M. A. Quijano and M. M. Bonilla, *Anal. Chim. Acta*, 2012, **714**, 38–46.
132 S. D. Conklin, N. Shockey, K. Kubachka, K. D. Howard and M. C. Carson, *J. Agric. Food Chem.*, 2012, **60**(37), 9394–9404.
133 K. E. Nachman, G. Raber, K. A. Francesconi, A. Navas-Acien and D. C. Love, *Sci. Total Environ.*, 2012, **417**, 183–188.
134 B. L. Batista, L. R. Nacano, S. S. De Souza and F. Barbosa, *Food Addit. Contam., Part A*, 2012, **29**(5), 780–788.
135 S. D. Conklin and P. E. Chen, *Food Addit. Contam., Part A*, 2012, **29**(8), 1272–1279.
136 A. A. Carbonell-Barrachina, X. C. Wu, A. Ramirez-Gandolfo, G. J. Norton, F. Burlo, C. Deacon and A. A. Meharg, *Environ. Pollut.*, 2012, **163**, 77–83.
137 T. Llorente-Mirandes, J. Calderon, J. F. Lopez-Sanchez, F. Centrich and R. Rubio, *Pure Appl. Chem.*, 2012, **84**(2), 225–238.
138 J. H. Huang, P. Fecher, G. Ilgen, K. N. Hu and J. Yang, *Food Chem.*, 2012, **130**(2), 453–459.
139 P. Alava, T. Van de Wiele, F. Tack and G. Du Laing, *Anal. Methods*, 2012, **4**(5), 1237–1243.
140 T. Narukawa, A. Hioki and K. Chiba, *J. Agric. Food Chem.*, 2012, **60**(4), 1122–1127.
141 T. Oguri, J. Yoshinaga, H. Tao and T. Nakazato, *Food Chem. Toxicol.*, 2012, **50**(8), 2663–2667.
142 B. P. Jackson, V. F. Taylor, M. R. Karagas, T. Punshon and K. L. Cottingham, *Environ. Health Perspect.*, 2012, **120**(5), 623–626.
143 L. B. Melendez, E. V. Silva, N. Miekeley, F. D. Vieira and S. M. Sella, *J. Braz. Chem. Soc.*, 2011, **22**(10), 1961–1967.
144 B. Marquez-Garcia, R. Perez-Lopez, M. J. Ruiz-Chancho, J. F. Lopez-Sanchez, R. Rubio, M. M. Abreu, J. M. Nieto and F. Cordoba, *J. Geochem. Explor.*, 2012, **119**, 51–59.
145 M. Button, M. M. Moriarty, M. J. Watts, J. Zhang, I. Koch and K. J. Reimer, *Chemosphere*, 2011, **85**(8), 1277–1283.
146 K. Torrance, H. Keenan, L. Munk and B. Hagedorn, *Environ. Geochem. Health*, 2012, **34**(6), 711–723.
147 I. Pizarro, D. Roman-Silva, C. Solar, M. M. Gomez, C. Camara and M. A. Palacios, *J. Chil. Chem. Soc.*, 2012, **57**(2), 1181–1188.
148 H. R. Farnfield, A. L. Marcilla and N. I. Ward, *Sci. Total Environ.*, 2012, **433**, 371–378.
149 M. A. Rahman, H. Hasegawa and R. P. Lim, *Environ. Res.*, 2012, **116**, 118–135.
150 M. A. Rahman and H. Hasegawa, *Appl. Geochem.*, 2012, **27**(1), 304–314.
151 V. W. M. Lai, K. Kanaki, S. A. Pergantis, W. R. Cullen and K. J. Reimer, *J. Environ. Monit.*, 2012, **14**(3), 743–751.
152 K. J. Whaley-Martin, I. Koch, M. Moriarty and K. J. Reimer, *Environ. Sci. Technol.*, 2012, **46**(6), 3110–3118.
153 M. Hamdi, M. Yoshinaga, C. Packianathan, J. Qin, J. Hallauer, J. R. McDermott, H. C. Yang, K. J. Tsai and Z. J. Liu, *Toxicol. Appl. Pharmacol.*, 2012, **262**(2), 185–193.
154 V. F. Taylor, B. P. Jackson, M. R. Siegfried, J. Navratilova, K. A. Francesconi, J. Kirshtein and M. Voytek, *Environ. Chem.*, 2012, **9**(2), 130–138.
155 A. Price, W. Maher, J. Kirby, F. Krikowa, E. Duncan, A. Taylor and J. Potts, *Environ. Chem.*, 2012, **9**(1), 77–88.
156 K. C. Hsu, C. C. Sun and Y. L. Huang, *Kaohsiung J. Med. Sci.*, 2011, **27**(9), 382–389.
157 J. M. Currier, M. Svoboda, T. Matousek, J. Dedina and M. Styblo, *Metallomics*, 2011, **3**(12), 1347–1354.
158 P. Alava, F. Tack, G. Du Laing and T. Van de Wiele, *Biomed. Chromatogr.*, 2012, **26**(4), 524–533.

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159 A. M. de Bettencourt, M. F. Duarte, M. H. Florencio, F. F. Henriques, P. A. Madeira, I. P. A. Maria and L. F. Vilas-Boas, *Microchem. J.*, 2011, **99**(2), 218–234.
160 D. P. Moraes, M. Svoboda, T. Matousek, E. M. M. Flores and J. Dedina, *J. Anal. At. Spectrom.*, 2012, **27**(10), 1734–1742.
161 C. T. Su, H. C. Lin, C. S. Choy, Y. K. Huang, S. R. Huang and Y. M. Hsueh, *Sci. Total Environ.*, 2012, **414**, 152–158.
162 J. Scheer, S. Findenig, W. Goessler, K. A. Francesconi, B. Howard, J. G. Umans, J. Pollak, M. Tellez-Plaza, E. K. Silbergeld, E. Guallar and A. Navas-Acien, *Anal. Methods*, 2012, **4**(2), 406–413.
163 A. Castillo, C. Boix, N. Fabregat, A. F. Roig-Navarro and J. A. Rodriguez-Castrillon, *J. Anal. At. Spectrom.*, 2012, **27**(2), 354–358.
164 F. Cubadda, F. Aureli, M. D'Amato, A. Raggi, A. C. Turco and A. Mantovani, *Pure Appl. Chem.*, 2012, **84**(2), 203–214.
165 N. Iriyama, Y. Yoshino, B. Yuan, A. Horikoshi, Y. Hirabayashi, Y. Hatta, H. Toyoda and J. Takeuchi, *J. Hematol. Oncol.*, 2012, **5**, 11.
166 J. Namiesnik and A. Rabajczyk, *Crit. Rev. Environ. Sci. Technol.*, 2012, **42**(4), 327–377.
167 R. Rakhunde, L. Deshpande and H. D. Juneja, *Crit. Rev. Environ. Sci. Technol.*, 2012, **42**(7), 776–810.
168 X. W. Zhao, N. Z. Song, W. H. Zhou and Q. Jia, *Cent. Eur. J. Chem.*, 2012, **10**(4), 927–937.
169 B. Novotnik, T. Zuliani, J. Scancar and R. Milacic, *J. Anal. At. Spectrom.*, 2012, **27**(9), 1484–1493.
170 M. M. L. Guerrero, E. V. Alonso, J. M. C. Pavon, M. T. S. Cordero and A. G. de Torres, *J. Anal. At. Spectrom.*, 2012, **27**(4), 682–688.
171 S. Dadfarnia, A. M. H. Shabani, M. R. Shishebor and K. H. Cisakht, *Int. J. Environ. Anal. Chem.*, 2011, **91**(13), 1320–1328.
172 P. R. Aranda, E. Perino, F. A. Bertolino, J. Raba and I. E. De Vito, *Microchim. Acta*, 2012, **179**(3–4), 235–239.
173 A. Bartyzel and E. M. Cukrowska, *Anal. Chim. Acta*, 2011, **707**(1), 204–209.
174 I. Lopez-Garcia, Y. Vicente-Martinez and M. Hernandez-Cordoba, *J. Anal. At. Spectrom.*, 2012, **27**(5), 874–880.
175 S. Sadeghi and A. Z. Moghaddam, *Talanta*, 2012, **99**, 758–766.
176 B. Majidi and F. Shemirani, *Microchim. Acta*, 2012, **176**(1–2), 143–151.
177 J. A. Baig, A. Hol, A. Akdogan, A. A. Kartal, U. Divrikli, T. G. Kazi and L. Elci, *J. Anal. At. Spectrom.*, 2012, **27**(9), 1509–1517.
178 H. M. Marwani, H. M. Albishri, E. M. Soliman and T. A. Jalal, *J. Dispersion Sci. Technol.*, 2012, **33**(4), 549–555.
179 M. Soylak and A. Aydin, *J. Iran. Chem. Soc.*, 2012, **9**(3), 263–267.
180 A. Moghimi, *Eur. J. Chem.*, 2011, **8**(3), 1052–1061.
181 J. Dai, F. L. Ren and C. Y. Tao, *Int. J. Environ. Res. Public Health*, 2012, **9**(5), 1757–1770.
182 Y. G. Abou El-Reash, M. Otto, I. M. Kenawy and A. M. Ouf, *Int. J. Biol. Macromol.*, 2011, **49**(4), 513–522.
183 D. Gladtke, A. Olschewski and U. Pfeffer, *Gefahrstoffe – Reinhalt. Luft*, 2012, **72**(5), 221–226.
184 B. Mongkhonsin, W. Nakbanpote, I. Nakai, A. Hokura and N. Jearanaikoon, *Environ. Exp. Bot.*, 2011, **74**, 56–64.
185 J. P. Thyssen, M. Strandesen, P. B. Poulsen, T. Menne and J. D. Johansen, *Contact Dermatitis*, 2012, **66**(5), 279–285.
186 D. J. Burbridge, I. Koch, J. Zhang and K. J. Reimer, *Chemosphere*, 2012, **89**(7), 838–843.
187 C. D. Pereira, J. G. Techy, E. M. Ganzarolli and S. P. Quinaia, *J. Environ. Monit.*, 2012, **15**(6), 1559–1564.
188 L. Telgmann, H. Faber, S. Jahn, D. Melles, H. Simon, M. Sperling and U. Karst, *J. Chromatogr., A*, 2012, **1240**, 147–155.
189 A. Albert, C. Brauckmann, F. Blaske, M. Sperling, C. Engelhard and U. Karst, *J. Anal. At. Spectrom.*, 2012, **27**(6), 975–981.
190 B. Meermann, M. Bockx, A. Laenen, C. Van Looveren, F. Cuyckens and F. Vanhaecke, *Anal. Bioanal. Chem.*, 2012, **402**(1), 439–448.
191 B. Meermann, A. Hulstaert, A. Laenen, C. Van Looveren, M. Vliegen, F. Cuyckens and F. Vanhaecke, *Anal. Chem.*, 2012, **84**(5), 2395–2401.
192 A. Izmer, D. Gholap, K. De Houwer, F. Cuyckens and F. Vanhaecke, *J. Anal. At. Spectrom.*, 2012, **27**(3), 413–418.
193 A. Moreda-Pineiro, V. Romaris-Hortas and P. Bermejo-Barrera, *J. Anal. At. Spectrom.*, 2011, **26**(11), 2107–2152.
194 V. Hansen, P. Yi, X. L. Hou, A. Aldahan, P. Roos and G. Possnert, *Sci. Total Environ.*, 2011, **412**, 296–303.
195 X. Han, L. H. Cao, H. Y. Cheng, J. H. Liu and Z. G. Xu, *Anal. Methods*, 2012, **4**(10), 3471–3477.
196 V. Romaris-Hortas, P. Bermejo-Barrera, J. Moreda-Pineiro and A. Moreda-Pineiro, *Anal. Chim. Acta*, 2012, **745**, 24–32.
197 R. Hirano, T. Nakagama, H. Nakajima, H. L. Zeng and K. Uchiyama, *Bunseki Kagaku*, 2012, **61**(9), 755–761.
198 S. Clemens, M. Monperrus, O. F. X. Donard, D. Amouroux and T. Guerin, *Talanta*, 2012, **89**, 12–20.
199 Y. Gao, Z. M. Shi, Z. Long, P. Wu, C. B. Zheng and X. D. Hou, *Microchem. J.*, 2012, **103**, 1–14.
200 S. Clemens, M. Monperrus, O. F. X. Donard, D. Amouroux and T. Guerin, *Anal. Bioanal. Chem.*, 2011, **401**(9), 2699–2711.
201 D. Esteban-Fernandez, M. Mirat, M. I. M. de la Hinojosa and J. I. G. Alonso, *J. Agric. Food Chem.*, 2012, **60**(34), 8333–8339.
202 Y. G. Yin, B. W. Chen, Y. X. Mao, T. Wang, J. F. Liu, Y. Cai and G. B. Jiang, *Chemosphere*, 2012, **88**(1), 8–16.
203 H. A. Zhu, X. D. Pan, J. L. Han, P. G. Wu, J. Tang and Y. Tan, *Food Addit. Contam., Part B*, 2012, **5**(3), 182–187.
204 Y. Q. Zhao, J. P. Zheng, L. Fang, Q. Lin, Y. N. Wu, Z. M. Xue and F. F. Fu, *Talanta*, 2012, **89**, 280–285.
205 P. Krystek, P. Favaro, P. Bode and R. Ritsema, *Talanta*, 2012, **97**, 83–86.
206 A. Krey, M. Kwan and H. M. Chan, *Environ. Res.*, 2012, **114**, 24–30.
207 J. J. B. Nevado, R. C. R. Martin-Doimeadios, F. J. G. Bernardo, N. R. Farinas and M. J. P. Ropero, *Talanta*, 2012, **99**, 859–864.

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208 P. Navarro, D. Amouroux, N. D. Thanh, E. Rochelle-Newall, S. Ouillon, R. Arfi, T. C. Van, X. Mari and J. P. Torreton, *Mar. Pollut. Bull.*, 2012, **64**(9), 1789–1798.
209 X. Y. Jia, D. R. Gong, Y. Han, C. Wei, T. C. Duan and H. T. Chen, *Talanta*, 2012, **88**, 724–729.
210 D. J. Kutscher, A. Sanz-Medel and J. Bettmer, *Metallomics*, 2012, **4**(8), 807–813.
211 Z. Pedrero, L. Ouerdane, S. Mounicou, R. Lobinski, M. Monperrus and D. Amouroux, *Metallomics*, 2012, **4**(5), 473–479.
212 C. Thiry, A. Ruttens, L. De Temmerman, Y. J. Schneider and L. Pussemier, *Food Chem.*, 2012, **130**(4), 767–784.
213 Y. Ogra, *Bunseki Kagaku*, 2012, **61**(6), 513–522.
214 F. Aureli, L. Ouerdane, K. Bierla, J. Szpunar, N. T. Prakash and F. Cubadda, *Metallomics*, 2012, **4**(9), 968–978.
215 S. Mechora, M. Germ and V. Stibilj, *Pure Appl. Chem.*, 2012, **84**(2), 259–268.
216 Y. Ohta, N. Suzuki, Y. Kobayashi and S. Hirano, *Isot. Environ. Health Stud.*, 2011, **47**(3), 330–340.
217 E. Kapolna, K. H. Laursen, S. Husted and E. H. Larsen, *Food Chem.*, 2012, **133**(3), 650–657.
218 K. L. Liu, Y. Zhao, F. S. Chen, Z. X. Gu and G. H. Bu, *Eur. Food Res. Technol.*, 2012, **234**(1), 61–68.
219 Q. L. Chan and J. A. Caruso, *Anal. Bioanal. Chem.*, 2012, **403**(5), 1311–1321.
220 G. S. Banuelos, S. S. Walse, S. I. Yang, I. J. Pickering, S. C. Fakra, M. A. Marcus and J. L. Freeman, *Anal. Chem.*, 2012, **84**(14), 6024–6030.
221 J. R. V. Barillas, C. F. Quinn, J. L. Freeman, S. D. Lindblom, S. C. Fakra, M. A. Marcus, T. M. Gilligan, E. R. Alford, A. L. Wangeline and E. A. H. Pilon-Smits, *Plant Physiol.*, 2012, **159**(4), 1834–1844.
222 V. Funes-Collado, R. Rubio and J. F. Lopez-Sanchez, *Water, Air, Soil Pollut.*, 2011, **222**(1–4), 315–324.
223 J. L. Stroud, S. P. McGrath and F. J. Zhao, *Int. J. Environ. Anal. Chem.*, 2012, **92**(2), 222–236.
224 H. Preud'homme, J. Far, S. Gil-Casal and R. Lobinski, *Metallomics*, 2012, **4**(5), 422–432.
225 V. Gomez-Jacinto, T. Garcia-Barrera, I. Garbayo, C. Vilchez and J. L. Gomez-Ariza, *Pure Appl. Chem.*, 2012, **84**(2), 269–280.
226 M. Sanchez-Martinez, E. G. P. da Silva, T. Perez-Corona, C. Camara, S. L. C. Ferreira and Y. Madrid, *Talanta*, 2012, **88**, 272–276.
227 W. Krittaphol, P. A. Wescombe, C. D. Thomson, A. McDowell, J. R. Tagg and J. P. Fawcett, *Biol. Trace Elem. Res.*, 2011, **144**(1–3), 1358–1369.
228 Y. Anan, S. Tanabe and Y. Ogra, *J. Toxicol. Sci.*, 2011, **36**(6), 725–731.
229 L. K. Gong, Q. B. Xu, C. Lee and H. Zhang, *Eur. Food Res. Technol.*, 2012, **235**(1), 169–176.
230 S. Yoshida, M. Haratake, T. Fuchigami and M. Nakayama, *Chem. Pharm. Bull.*, 2012, **60**(3), 348–353.
231 D. T. Juniper, R. H. Phipps and G. Bertin, *Animal*, 2011, **5**(11), 1751–1760.
232 M. Malinouski, S. Kehr, L. Finney, S. Vogt, B. A. Carlson, J. Seravalli, R. Jin, D. E. Handy, T. J. Park, J. Loscalzo, D. L. Hatfield and V. N. Gladyshev, *Antioxid. Redox Signaling*, 2012, **16**(3), 185–192.
233 M. Malavolta, F. Piacenza, A. Basso, R. Giacconi, L. Costarelli, S. Pierpaoli and E. Mocchegiani, *Anal. Biochem.*, 2012, **421**(1), 16–25.
234 G. Ballihaut, L. E. Kilpatrick and W. C. Davis, *Anal. Chem.*, 2011, **83**(22), 8667–8674.
235 J. Bianga, G. Ballihaut, C. Pecheyran, Z. Touat, H. Preud'homme, S. Mounicou, L. Chavatte, R. Lobinski and J. Szpunar, *J. Anal. At. Spectrom.*, 2012, **27**(1), 25–32.
236 Y. Suzuki, T. Sakai and N. Furuta, *Anal. Sci.*, 2012, **28**(3), 221–224.
237 M. Dernovics, A. Vass, A. Nemeth and A. Magyar, *Talanta*, 2012, **99**, 186–193.
238 Y. Lu, A. Rumpler, K. A. Francesconi and S. A. Pergantis, *Anal. Chim. Acta*, 2012, **731**, 49–59.
239 B. Gammelgaard, S. Sturup and M. V. Christensen, *Metallomics*, 2012, **4**(2), 149–155.
240 X. J. Mao, B. Hu, M. He and B. B. Chen, *J. Chromatogr., A*, 2012, **1256**, 32–39.
241 P. K. Petrov, J. W. Charters and D. Wallschlager, *Environ. Sci. Technol.*, 2012, **46**(3), 1716–1723.
242 H. Akiho, T. Yamamoto, Y. Tochihara, N. Noda, S. Noguchi and S. Ito, *Fuel*, 2012, **102**, 156–161.
243 C. F. Harrington, R. Clough, H. R. Hansen, S. J. Hill and J. F. Tyson, *J. Anal. At. Spectrom.*, 2010, **25**(8), 1185–1216.
244 C. F. Harrington, R. Clough, L. R. Drennan-Harris, S. J. Hill and J. F. Tyson, *J. Anal. At. Spectrom.*, 2011, **26**(8), 1561–1595.
245 N. Campillo, P. Vinas, R. Penalver, J. I. Cacho and M. Hernandez-Cordoba, *J. Food Compos. Anal.*, 2012, **25**(1), 66–73.
246 J. P. Barnett, D. J. Scanlan and C. A. Blindauer, *Anal. Bioanal. Chem.*, 2012, **402**(10), 3311–3322.
247 J. P. Barnett, D. J. Scanlan and C. A. Blindauer, *Anal. Bioanal. Chem.*, 2012, **402**(10), 3371–3377.
248 A. Szterk, M. Roszko, K. Malek, M. Czerwonka and B. Waszkiewicz-Robak, *Meat Sci.*, 2012, **91**(4), 408–413.
249 P. Kodali, K. R. Chitta, J. A. L. Figueroa, J. A. Caruso and O. Adeoye, *Metallomics*, 2012, **4**(10), 1077–1087.
250 R. N. Easter, C. G. Barry, G. Pyne-Geithman and J. A. Caruso, *Metallomics*, 2012, **4**(1), 48–55.
251 L. R. V. Mataveli, M. Fioramonte, F. C. Gozzobc and M. A. Z. Arruda, *Metallomics*, 2012, **4**(4), 373–378.
252 R. Liu, Y. Lv, X. D. Hou, L. Yang and Z. Mester, *Anal. Chem.*, 2012, **84**(6), 2769–2775.
253 T. Konz, M. Montes-Bayon and A. Sanz-Medel, *Anal. Chem.*, 2012, **84**(19), 8133–8139.
254 S. Crotti, C. Granzotto, W. R. L. Cairns, P. Cescon and C. Barbante, *J. Mass Spectrom.*, 2011, **46**(12), 1297–1303.
255 S. J. Christopher, E. L. Kilpatrick, L. L. Yu, W. C. Davis and B. M. Adair, *Talanta*, 2012, **88**, 749–758.
256 L. Waentig, N. Jakubowski, S. Hardt, C. Scheler, P. H. Roos and M. W. Linscheid, *J. Anal. At. Spectrom.*, 2012, **27**(8), 1311–1320.
257 U. Bergmann, R. Ahrends, B. Neumann, C. Scheler and M. W. Linscheid, *Anal. Chem.*, 2012, **84**(12), 5268–5275.

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258 D. Esteban-Fernandez, R. Ahrends and M. W. Linscheid, *J. Mass Spectrom.*, 2012, **47**(6), 760–768.
259 D. Esteban-Fernandez, F. S. Bierkandt and M. W. Linscheid, *J. Anal. At. Spectrom.*, 2012, **27**(10), 1701–1708.
260 Y. R. Tang, X. Jiao, R. Liu, L. Wu, X. D. Hou and Y. Lv, *J. Anal. At. Spectrom.*, 2011, **26**(12), 2493–2499.
261 P. Jarujamrus, R. Chawengkirttikul, J. Shiowatana and A. Siripinyanond, *J. Anal. At. Spectrom.*, 2012, **27**(5), 884–890.
262 Y. Li, S. K. Sun, J. L. Yang and Y. Jiang, *Analyst*, 2011, **136**(23), 5038–5045.
263 D. Hare, C. Austin and P. Doble, *Analyst*, 2012, **137**(7), 1527–1537.
264 I. Konz, B. Fernandez, M. L. Fernandez, R. Pereiro and A. Sanz-Medel, *Anal. Bioanal. Chem.*, 2012, **403**(8), 2113–2125.
265 C. Giesen, T. Mairinger, L. Khoury, L. Waentig, N. Jakubowski and U. Panne, *Anal. Chem.*, 2011, **83**(21), 8177–8183.
266 D. J. Kutscher, M. B. Fricker, B. Hattendorf, J. Bettmer and D. Gunther, *Anal. Bioanal.Chem.*, 2011, **401**(9), 2691–2698.
267 E. Moreno-Gordaliza, D. Esteban-Fernandez, C. Giesen, K. Lehmann, A. Lazaro, A. Tejedor, C. Scheler, B. Canas, N. Jakubowski, M. W. Linscheid and M. M. Gomez-Gomez, *J. Anal. At. Spectrom.*, 2012, **27**(9), 1474–1483.
268 J. Y. Joo and H. B. Lim, *J. Anal. At. Spectrom.*, 2012, **27**(7), 1069–1073.

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