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Review

Elemental speciation by chromatographic separation with inductively coupled plasma mass spectrometry detection

Claudia A. Ponce de León, Maria Montes-Bayón, Joseph A. Caruso*

Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221-0172, USA

Abstract

Separation techniques coupled to inductively coupled plasma mass spectrometry (ICP-MS) is reviewed. ICP-MS technique is described briefly. Coupling of the different separation techniques are described, together with the most common applications used for each technique that has been described in the literature. An overview for the future of separation techniques coupled to ICP-MS with regard to elemental speciation is discussed.

Keywords: Reviews; Inductively coupled plasma mass spectrometry; Elemental speciation

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1. Introduction

Many critical processes in biological systems and in the environment require metals and their importance has been recognized for over 100 years. To begin understanding the role of metals in ecological

*Corresponding author. Tel.: +1-513-5565-858; fax: +1-513-556-0142.

E-mail address: joseph.caruso@uc.edu (J.A. Caruso).

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systems, different requirements must be fulfilled. Metals must be detected with high specificity and selectivity; their quantification must be obtained with high accuracy and sometimes at very low levels. However, in assessing the role of metals in biological systems, the presence and the amount of metal may give little information about its risk or benefit. To effectively assess these parameters it is necessary to elucidate the chemical form of such metals in the sample, be they inorganic or organic forms (species). This practice of determining the different species is known as elemental speciation analysis or metal speciation analysis. As more systems involving metals have been discovered, the number of publications regarding the metal speciation and analyses has increased.

Speciation analysis requires the implementation of different analytical techniques to correctly determine the metal species. Inductively coupled plasma mass spectrometry (ICP-MS) has swiftly become the preferred technique to couple with chromatographic separations. It is versatile and reliable in terms of detection limits ($\leq$ppt levels), linearity (up to 10^8 orders of magnitude), and specificity (the ability to monitor one or more *masses* at the same time without overlapping elemental signals). ICP-MS also presents some difficulties such as interferences on the monitored mass. These interferences can be isobaric (from molecular species), caused by atomic or molecular species that have the same mass as the analyte of interest; and the nonspectral interferences given mostly by the sample matrix. The latter interferences include variations in the analyte transport to the plasma, extraction of the ions from the interface, or defocusing of the ion beam due to electrostatic repulsion.

Isobaric interferences may be minimized by the use of mixed gases (Xe, He, etc.), utilization of alternative plasmas (e.g., low pressure or cold plasmas), or utilization of reagents that minimize the formation of interfering polyatomic ions.

When a chromatographic technique is used in conjunction with ICP-MS, it adds a dimension of selectivity by separating the individual elemental species and presenting them in sequence to the detector. One of the weaknesses of the coupling of a chromatographic technique with ICP-MS is the lack of structural information for the species of interest. It is necessary then to use standards to match retention times. But then one is presented with the shortage of reference materials or standards for the elemental species in the study. These standards and reference materials are necessary for identification and validation of the studied species.

Nevertheless, chromatography with ICP-MS detection still offers the advantages of high sensitivity and selectivity and a simple configuration that has made this coupling the methodology of choice for elemental speciation.

2. Inductively coupled plasma mass spectrometry

Plasmas have been defined as highly ionized gases in which the number of free electrons is approximately equal to the number of positive ions. The inductively coupled plasma is a special type of plasma produced in a gas (usually argon) at atmospheric pressure, sustained by inductively coupling energy from a high frequency field. The field is typically applied by an induction coil with an ac current at frequencies of about 30 MHz and power levels of 1000–2000 W. The temperature reached in the plasma ranges from 6000 to 10 000 K, which is suitable for the atomization excitation and ionization of elemental species [1–3]. The quartz torch where the plasma is contained consists of three concentric tubes into which different argon flows are introduced. These flows create three main components of the plasma: the external channel acting as the coolant gas and plasma support gas, the internal channel preventing the plasma from diffusing or collapsing downward and the central channel where samples are introduced as fine aerosols or gases. When samples are introduced into the plasma, they go through desolvation, vaporization, atomization and ionization processes before entering the mass analyzer.

There are many methods for introducing samples into the plasma, depending on whether or not they are solid, liquid or gaseous, although most systems are designed for liquid sample introduction. Conventional pneumatic nebulization is the most common way for liquid introduction and it is done through nebulizers, which include the concentric type, Babington, fritted disc micro channel plate, cross-flow

nebulizers and some direct injection types. The nebulization efficiency of different nebulizers varies between 1 and 3% though ultrasonic nebulizers enhance nebulization efficiency but require desolvation. More recent versions of ultrasonic nebulizers include a desolvation unit where aerosols are desolvated efficiently, but have the disadvantages of memory effects and peak broadening. The ions emerging from the ICP are extracted to the low pressure mass spectrometer interface through the sampling and skimmer cones (usually made of nickel). The ions are then focused into the mass analyzer using a series of ion lenses. The positively charged ions obtained from the ion lenses chamber are then separated according to their mass to charge ratio in the mass analyzer. The ions are typically detected by an electron multiplier, where the electron pulse is amplified.

The quadrupole has been the most widely used mass analyzer for ICP-MS. To achieve higher resolutions and thereby reduce isobaric interferences (such as $^{40}Ar^{35}Cl^{+}$ interfering with $^{75}As^{+}$), double focusing sector field mass analyzers may be used. The time-of-flight (TOF) mass analyzer is potentially very useful for speciation applications, since it is capable of very fast data acquisition, an attractive feature when dealing with rapidly sequencing narrow chromatographic peaks such as with capillary gas chromatography (GC) or capillary electrophoresis (CE). It also offers the capability of acquiring a full spectrum, even if very small volumes are used. When TOF mass analyzers are used with chromatographic systems, high precisions are attained when in isotope ratio mode.

Table 1
Typical ICP-MS operating conditions

Parameter	Instrument setting
Radio frequency forward power (W)	1350
Radio frequency reflected power (W)	<5
Plasma gas flow (l/min)	16
Auxiliary gas flow (l/min)	0.8–1.1
Nebulizer gas flow-rate (l/min)	0.7–1.0
Liquid sample flow-rate (ml/min)	1.0
Sample introduction system	
Nebulizer	Concentric
Spray chamber	Scott-type double pass
Pump settings	
Expansion stage pressure	$<2\times10^{\circ}$
Intermediate stage pressure	4×10^{-6}
Analyzer stage pressure	0.0×10^{-4}

Typical ICP-MS operating conditions for liquid sample introduction are shown in Table 1. These conditions will vary especially when ICP-MS is coupled to GC or supercritical fluid chromatography.

3. Chromatographic techniques

Elemental speciation, as already mentioned, requires coupling a separation technique with ICP-MS detection. Selection of the specific chromatographic technique will depend on the nature of the sample and the resources at hand. Szpunar et al. [4] indicate clearly how different chromatographic techniques can be used for the same analysis and be adapted for particular needs. Each separation technique has its own advantages and special requirements when interfaced with ICP-MS. Good discussions on interfacing to ICP-MS can be found in Caruso et al. [5].

3.1. Liquid chromatography

High-performance liquid chromatography (HPLC) is the chromatographic technique most widely used with ICP-MS detection, since once the properly prepared sample is ready for analysis; no additional sample treatment is needed. Chemical properties such as polarity, solubility, ionic charge and size, and molecular mass of the species to be analyzed will determine the type of liquid chromatography to be used.

Coupling HPLC with ICP-MS is straightforward. Usually PEEK tubing is connected from the outlet of the HPLC column to the inlet of the nebulizer. Some precautions should be observed for good chromatographic resolution, such as keeping the transfer line to a minimal length and with a minimal internal diameter to avoid peak broadening. Also, improper interface design and usage may decompose and/or alter the species of interest.

Acon et al. [6] describe an interface for microbore HPLC systems. A DIHEN nebulizer (direct injection high efficiency nebulizer) was interfaced to the ICP-MS by inserting into a Delrin® adapter and the nebulizer gas flow was controlled using a mass flow controller. With this interface, the dead volume of the

nebulizer was reduced to approximately 10 μl, therefore, reducing the peak broadening by 3 s. They also were able to introduce 20% acetonitrile into the plasma. The different nebulizers available for ICP-MS also have an impact on the determination of species eluting from HPLC columns. Ackley et al. [7] compared the performance of concentric, microconcentric and micro-mist nebulizers when used to interface microbore LC columns with methanol mobile phases. When the mobile phase contained 20% methanol or more, the analyte signal was independent of sample flow-rate. Micro mist nebulizers performed better when the mobile phase contained 70% methanol compared to 20% methanol. Gammelgaard and Jons [8] evaluated an ultrasonic nebulizer (USN) and a cross-flow nebulizer for selenium speciation analyses with ion-chromatography. They obtained better sensitivities for all Se species with the USN, although the sensitivity was different and dependent on pH and USN temperature for the different Se species.

Oscillating capillary (OCN) and high efficiency nebulizers (HEN) adapted into either a single pass, double pass and cyclonic spray chamber for minibore columns were evaluated by B'Hymer et al. [9]. The combination HEN-cyclonic spray chamber gave better detection limits, while OCN with any of the spray chambers gave worse detection limits. Evaluation of different spray chambers was done in 1996 by Rivas et al. [10].

The major problem in coupling ICP-MS with HPLC is a reduction in sensitivity due to the high saline content [11], which tends to deposit in the sample skimmer cones of the MS interface, or the organic solvents used in the mobile phase, which produce carbon deposits. Different approaches have been tried to overcome these problem. These include the substitution of sodium or potassium salts by ammonium salts in the buffer, keeping the buffer dissolved solids below 0.1%, or the addition of oxygen (approximately 10%, v/v) to the nebulizer gas to minimize carbon deposits from the organic solvents. Cooling the spray chamber (0–5 °C) and increasing the RF power also contribute to lessening the carbon deposits in the cones. Larsen [12] discusses the influence of organic solvents on the element response for ICP-MS. He discusses how the different types of spray chambers and nebulizers are sensitive to gradient elutions, and how organic solvents can get trapped or adhere to the walls of the spray chamber gradually changing the signal for some elements such as As and Se.

Another problem is the low nebulization efficiency of the mobile phase at flow-rates of 1 ml/min. Olesik et al. [13] have reported transport efficiencies of up to 20% when liquid sample introduction is less than 0.1 ml/min, making small bore and microbore attractive options for HPLC, even though the total analyte to the plasma may be roughly the same.

An alternate introduction system prior to ICP-MS detection is the post column hydride generator. Hydride generation (HG) is a convenient introduction system for elements that form volatile hydrides such as arsenic, bismuth, germanium, lead, antimony, selenium, tin and tellurium. These hydrides are efficiently transported to the plasma, which results in better detection limits when compared to solution nebulization.

Hydride generation can be carried post column utilizing different chromatographic techniques and a variety of samples. Baby food has been analyzed by ion-exchange chromatography for arsenic compounds [14], cadmium metallothioneins in fish cytosols has been analyzed by vesicle-mediated HPLC [15], arsenic speciation has been achieved by ion-exclusion chromatography and validated with human urine and tuna fish tissue reference materials [16], methyl mercury detection on fresh water humic and fulvic acids with gel permeation chromatography was achieved by O'Driscoll and Evans [17]. Fig. 1 shows the schematic diagram of the vesicle-mediated HPLC-HG-ICP-MS system, whose structure is similar to other post column hydride generation systems. Elements such as selenium and arsenic have been continuously speciated in different sample matrices with post column hydride generation [18–24]. These elements are of great health importance and present isobaric interferences for their main isotopes; hydride generation presents an alternative for better detection limits.

Almost all modes of liquid chromatography can be coupled to ICP-MS. Reversed-phase, reversed-phase ion-pairing, ion-exchange, chiral, and size exclusion are those that have had primary application with ICP-MS detection. Capillary electrophoresis (CE) also has received wide attention as a separation

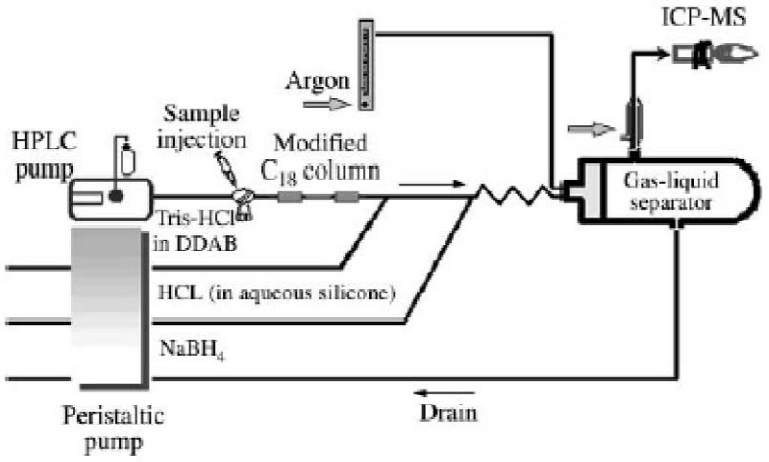

Fig. 1. HPLC–ICP-MS chromatograms showing the separation of different seleno compounds utilizing three different ion-pair reagents (0.1% TFA, PFPA and HFBA). From Ref. [27] with permission of J. Chromatogr. A.

technique with ICP-MS detection. Although the coupling is uncomplicated, the connections suffer from low sensitivity (due to low transfer efficiency) and band broadening (due to large dead volumes) causing poor selectivity.

3.1.1. Reversed phase chromatography

In this type of chromatography, the analytes are separated in the chromatographic column according to their affinity for the stationary phase or mobile phase, where the stationary phase is less polar than the mobile phase. Typically reversed-phase separations use siloxane C_{18}, C_8 and C_2 stationary phases. With these stationary phases, mobile phases of extreme pH must be avoided since degradation or solubilization of the packing material occurs. Alternately, polymeric stationary phases may be utilized for elemental speciation studies. These provide stability over a wider pH range. Metal speciation depends very closely on the separation attained with the chromatographic system, even same phase columns may perform differently [25] and ultimately affect the speciation.

The separation may be controlled by varying the amount and type of eluents in the mobile phase. Methanol solutions are the most widely used mobile phases when coupled to an ICP, since it causes less plasma instability [26]. Due to the plasma instability with organic solvents, applications of reversed-phase chromatography with ICP-MS detection are limited.

3.1.2. Reversed-phase ion-pairing chromatography

In this type of chromatography, a counter ion is added to the mobile phase of the conventional reversed-phase system, and has the advantage of separating charged and uncharged molecular species. The counter-ion is typically referred to as the ion-pair reagent; consisting of a polar head and a non-polar tail. Commonly used ion-pairing reagents are C_5 or greater alkyl ions (e.g., tetraalkylammonium or alkylsulfonate salts). The polar head of the ion-pairing reagent binds to the ionic molecule to form an electrically neutral ion-pair, which is then retained by the reversed-phase column. The counter ions used should be soluble in the mobile phase, univalent, aprotic and non-destructive to the stationary phase. Typical concentrations for the ion pairing reagents range from 1 to 5 m*M*. A work that exemplifies the influence of the ion-pair reagents over a chromatographic separation is seen in the study of Kotrebai et al. [27]. They present the separation of 13 selenocompounds with three different ion-pair reagents. These ion-pair reagents are suitable for the coupling of ICP-MS detection because their volatility is easily handled by the ICP. The chromatograms are presented in Fig. 1.

Table 2 shows some speciation analyses made

Table 2
Specific applications of reversed-phase ion-pair LC with ICP-MS detection

Element(s) analyzed	Species analyzed	Sample type	Chromatography	Detector	Notes	Reference
As	Arsenite, arsenate, monomethylarsonic acid, dimethylarsinic acid, arsenobetaine and arsenocholine	Spring waters and bottled mineral waters	Hamilton PRP1 resin-based, reversed-phase column, 250×4.6 mm. Mobile phase 0.5 m*M* tetrabutylammonium phosphate, pH buffered with 4 m*M* Na_2HPO_4 and adjusted to pH 9 with ammonia	VG Elemental PlasmaQuad II+ with concentric nebulizer and double pass spray chamber. Acquisition of data in both scanning and peak jump modes	Detection limits in the range 1.0–3.0 μg l^{-1}. Good mass balance obtained with a hydride generation method for total As	[134]
As	Arsenite, arsenate, monomethylarsonic acid, dimethylarsinic acid, arsenobetaine, arsenocholine	Urine, after digestion of sea-food samples	Phenomenex μBondclone reversed-phase C_{18} column, 300×3.9 mm or Waters Bondpack C_{18} 300 mm×3.9 mm column. Various ion pairing reagent and mobile phase compositions evaluated	VG PlasmaQuad II+ with Meinhard nebulizer and cooled spray chamber (4 °C)	Mixed ion-pairing reagents used (10 m*M* hexanesulfonate and 1 m*M* tetraethylammonium hydroxide. Complete separation in 12 min of all species	[21]
As	As species in animal feed additives	Environmental and biological reference materials	RP micro-HPLC used. Isco Spherisorb 3 μm C_{18} material, 150×1 mm I.D. Mobile phase 0.1% TFA and 5–10% MeOH in water. TBAH (1–5 m*M*) ion-pairing reagent. Flow-rate 15–40 μl min^{-1}	VG PlasmaQuad II STE	70% of total arsenobetaine ingested was excreted into urine. Reversed phase chromatography minimized co-elution of species. Low flow-rates minimized waste. Limits of detection in sub-pg range	[135]
As	Arsenobetaine in NIES in candidate certified reference material	Human urine	Comparison of reversed-phase ODS, cation-exchange silica-based LCSCX and styrene–divinylbezene copolymer-based PRP–X-100 and gel permeation columns.	Hydride generation and ICP-MS detection	Determination of several As species. Special attention to Arsenobetaine for reference material CRM no. 18.	[136]
As	Arsenite, demethylarsinic, monomethyl arsonic and arsenate	Wine and kelp samples	Octadecyldimethylsilyl reversed-phase narrow-bore HPLC column. Mobile phase containing 5 mm tetrabutylammonium hydroxide as the ion-pairing reagent at pH 6	ICP-MS detection	Recoveries of the As species ranged from 92 to 98% in spiked samples. Limits of detection on the low ppb range	[137]

Pb	Inorganic lead, triethyllead chloride, triphenyllead chloride, tetraethyllead	Lead in reference fuel and water	Nucleosil C_{18} 5 μm (250×4.6 mm I.D.). Mobile phase 8 m*M* PIC-B5 at pH 3. Gradient elution 40–90% methanol in water over 10 min, held at 90% methanol for 20 min	VG PlasmaQuad. Forward power 1.40 kW. Nebulizer gas flow 0.68 l min^{-1}. Concentric nebulizer with double pass spray chamber cooled to −10 °C	Detection limits in range 0.37–3.9 ng for the various species	[138]
Pb	Inorganic lead, trimethyllead and triethyllead		5 μm Hypersil ODS column, gradient elution of 10:90 to 30:70 methanol:buffer eluent. Buffer prepared from 0.1 *M* sodium acetate, 0.1 *M* acetic acid and 4 m*M* sodium pentanesulfonate	Single pass 40-ml spray chamber and concentric nebulizer used to minimize dead volume. Isotope dilution measurements made	Long term stability of Et_3PbCl questioned. Data acquisition made complicated by lack of suitable software	[139]
Hg	Inorganic mercury and methylmercury		Four different ODS columns evaluated. Various mobile phases evaluated all containing 10 m*M* tetrabutylammonium bromide	Perkin-Elmer Sciex Elan 5000A with a cooled double pass spray chamber and a cross-flow nebulizer	The method was unsuccessful as Hg was absorbed by the ODS column, leading to a slow bleed of mercury. Use of non-stainless steel components and glass-lined columns reduced these problems	[140]
Hg	Inorganic mercury, methylmercury and methylmercury	Open ocean sea water reference material and tap water	Spherisorb ODS-2 (150×4.6 mm i.d) column. Mobile phase 0.5% (m/v) L-cysteine, pH 5 at flow-rate of 1.6 ml min^{-1}	Perkin-Elmer ELAN 5000. Samples introduced using in situ vapor generation	Cold vapor generation with LC–ICP-MS found to be better than conventional pneumatic nebulizer in terms of sensitivity	[141]
Se/As	Thirteen selenium and arsenic species	Canned tuna fish and human urine	Phenomenex reversed-phase C_{18} (250×4.6 mm) with guard column. 10 m*M* sulfonate and 0.1% methanol mobile phase, pH 3.5 at 1.0 ml min^{-1}	VG Elemental PQ2+ Turbo with Meinhard concentric nebulizer and cooled spray chamber (4 °C)	*m/z* 75, 77 and 78 enabled simultaneous monitoring of arsenic, selenium and potential interfering species	[142]
Se	Selenoamino acids	Selenium enriched yeast	Zorbax SB-C_8 (150×4.6 mm) and guard column (12.5×4.6 mm). Mobile phase methanol:water (98:2), 0.1% (v/v) trifluoroacetic acid	Perkin-Elmer SCIEX ELAN ICP-MS with cross-flow nebulizer and double pass spray chamber	Presence of selenocystine, selenomethionine and methylselenocysteine confirmed	[143]
Se	Se (IV), Se(VI) and selenoamino acids	Selenium nutritional supplements	Phenomenex C_8 (250×4.6 mm) And guard column. Mobile phase 10% MeOH and 1% trifluoroacetic acid (TFA)	Perkin-Elmer ELAN 6000 ICP-MS with micro-concentric nebulizer	Several extraction methods (acid treatment, enzymatic hydrolysis) for total determination of selenium species in nutritional supplements	[29]
Se	Inorganic Se, selenoamino acids and trimethyl-selenonium	Selenium nutritional supplements	RP nucleosil 120 C_{18} (250×4.0 mm). Mobile phase 30 m*M* ammonium formate, pH 3.0, 5% (v/v) MeOH and also 10 m*M* TBAA (ion pairing)	ICP-MS (Platform ICP) from Micromass using collision and reaction cell with H_2	Evaluation of different nebulizers for introduction of HPLC eluents in a collision cell instrument. Comparison of reversed-phased with ion-pairing	[144]

Se	Inorganic Se and 23 different selenoamino acids	Selenized plants, selenized garlic and onion	5 μm Symmetry Shield RP8 (15×3.9 mm I.D.) which has a polar modifier group between the C_8 group and the silica base. Mobile phase 99:1 (v/v) water–methanol with (a) 0.1% TFA or (b) 0.1% HFBA	ICP-MS Elan 5000 and Elan 5000a. Meinhard nebulizer with an in-house fabricated spray chamber containing an impact bead	Evaluation of different perfluorinated ion pairing reagents (TFA and HFBA) for the resolution of 25 Se species in 70 min chromatogram	[27]
Se/S	Selenate, selenite, selenocystine, selenocystamine, selenomethinine, selenoethionine cysteine, cystine, cystamine, reduced glutathione and methionine	Human urine	RP nucleosil 120 C_{18} (250×4.0 mm). Mobile phase 30 m*M* ammonium formate, pH 3.0, 5% (v/v) MeOH and also 10 m*M* TBAA (ion pairing)	ICP-MS (Platform ICP) from Micromass using collision and reaction cell with H_2	Comparison of different human urines with separations by reversed-phase and ion-exchange	[28]
Sn	Tributyltin, triphenyltin	Waters	Kromasil-100 C_{18} column. Mobile phase 82% (v/v) methanol, 2.5% acetic acid, 0.3% tetraethylamine and 10 mg l^{-1} oxalic acid	Perkin-Elmer ELAN 5000A with double-skinned, silvered glass spray chamber at −15 °C. 1250 W forward power. Nebulizer gas flow 0.95 l min^{-1}	Preconcentration first performed on a Sep-Pak C_{18} minicolumn to obtain a detection limit of 2 ng l^{-1} for TBTCl	[145]
Sn	Trimethyl-, triphenyl- and tributyltin chlorides	Fish tissue	Silica and polymer stationary phase C_{18} columns compared. Sodium pentane sulfonate ion pairing reagent	VG PQ I with 1% argon addition to the nebulizer gas flow. A cooled (−20 °C) double pass spray chamber was used	Inorganic tin was held more strongly on the silica-based column. Extraction and recoveries were reasonable	[146]

Taken in part from Sutton and Caruso [133] with permission of J. Chromatogr. A.

with this technique. The more recent applications of reversed-phase ion-pairing chromatography involve selenium speciation. Gayon et al. [28] speciated human urine using RP nucleosil 120 C_{18} (250×4.0 mm). Mobile phases of 30 m*M* ammonium formate, pH 3.0, 5% (v/v) MeOH and also 10 m*M* TBAA (ion-pairing) have been used. To minimize matrix effects, a hexapole collision and reaction collision cell was optimized. B'Hymer and Caruso [29] analyzed yeast based supplement extracts from different digestion methods using a Phenomenex C_8 (250×4.6 mm) utilizing a mobile phase of 10% MeOH and 1% trifluoroacetic acid (TFA).

3.1.3. Micelle and vesicle-mediated chromatography

These two chromatographies are alternatives to reversed-phase liquid chromatography and offer the combination of a micellar media with the capability of an aqueous–organic mobile phase separation. The micelles or vesicles are obtained when the aqueous mobile phase is a surfactant solution at a concentration above the critical micellar concentration. Different types of interactions take place between solutes, micelles and stationary phase giving more versatility than conventional RP-HPLC. When the surfactant has two or more hydrophobic tails it can form bi-layers that if sonicated can form doughnut-shaped aggregates called vesicles. These vesicles are less dynamic than the micelles, conferring more stable aggregates, thus presenting more interaction sites.

This technique is appropriate for the analysis of physiological fluids since the mobile phase is capable of maintaining proteins in solution. LaFuente et al. analysed selenium species in urine [18] and Infante et al. [15] were able to identify cadmium metallothionein in eel liver and kidney cytosols. Fig. 2 shows the schematic diagram for the set-up HPLC–hydride generation-ICP-MS they used.

Tin compounds have been analysed by Suyani et al. [30] and Inoue et al. [31], while arsenic compounds have been studied by Ding et al. [32]. A review of micellar liquid chromatography for physiological fluids by Garcia and Broch [33], mentions the possibility of ICP-MS detection.

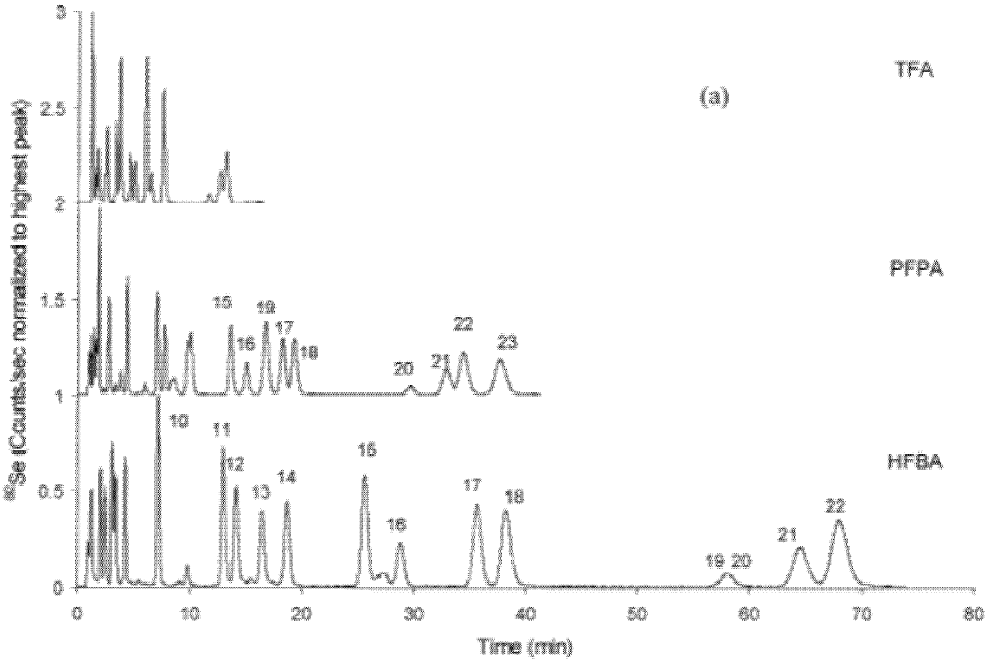

Fig. 2. Schematic diagram of the vesicle-mediated HPLC–ICP-MS System. Diagram obtained from Ref. [15], reproduced by permission of The Royal Society of Chemistry.

Table 3
Specific applications of ion-exchange LC with ICP-MS detection

Element(s) analyzed	Species analyzed	Sample type	Chromatography	Detector	Notes	Reference
As	As (III), As (V), dimethylarsinic acid, monomethylarsonic acid	Soil and sediment samples	Anion-exchange. Hamilton PRP-X100 anion-exchange column (250×4.6 mm). Mobile phase: $(NH_4)_2HPO_4$ 10–100 m*M*, 3% MeOH. Gradient elution at 1.0 ml min^{-1} flow-rate	VG PlasmaQuad II+. 1350 W rf power, nebulizer gas 0.80 l min^{-1}, glass concentric nebulizer and borosilicate glass double pass spray chamber (5 °C)	Recoveries of up to 80% of the total arsenic content were obtained. The detection limits were in the range 1–2 mg kg^{-1} for all species. Arsenate was found in the soil and arsenite in the sediment	[147]
As	As (III), As(V), methylarsonic acid, dimethylarsinic acid, arsenobetaine	Urine	Anion-exchange. Dionex IonPac AS14 4 mm. Mobile phase: 2 m*M* tetramethylammonium hydroxide, 10 m*M* ammonium carbonate, flow-rate 1.5 ml min^{-1}	Perkin-Elmer ELAN 5000, with Meinhard concentric nebulizer and cyclone spray chamber	To avoid matrix effects, the urine samples were diluted 1:5 with water and filtered	[49]
As	As (III), As(V), dimethylarsinate, mono-methylarsonate, arsenocholine and arsenobetaine	Mung bean seedlings, used as a bioindicator for arsenic contamination	Anion-exchange. Waters IC-PAK (75×4.6 mm). Mobile phase: 80% sodium dihydrogen phosphate, 20% sodium dihydrogen phosphate, pH 6 at 0.5 ml min^{-1}	VG PQII+, Nebulizer flow-rate of 0.9 ml	Most of the arsenic is accumulated in the roots. A reduction of As(V) to As(III) occurs in the roots	[51]
As	As (III), As(V), methylarsonic acid dimethylarsinic acid, arsenobetaine	Apple samples after different extraction procedures	Anion-exchange PRP-X100 columns (Hamilton). Mobile phase:10 m*M* ammonium phosphate and 10 m*M* ammonium nitrate at pH 6.2	VG PQII, Concentric nebulizer with a double pass spray chamber	100% recoveries of As species when the extraction is performed by amylase treatment/sonication extraction with 50:50 MeOH–water	[67]
Br	Bromate	Drinking waters	Anion-exchange. Dionex AG 10. Mobile phase: 100 m*M* NaOH. preconcentration also performed using this column	VG PQII. Ultrasonic nebulizer	Detection limits of 50 ppt for bromate obtained with ultrasonic nebulizer	[37]

Cr	Cr(III) and Cr(VI)	Aquatic samples	Anion-exchange. IC-Pak. Cation-exchange Guard-Pak CM/D (3.9×150 mm)	VG PQII with a concentric nebulizer, Scott type spray chamber (4 °C). Rf power 1350 W. Neb gas flow 0.80 l min^{-1}	Separate fraction divided into species retained on cation column, anion column and those that elute in void volume	[148]
Cr	Cr (III) and Cr(VI)		Anion-exchange. IonPac-AG5 (50×4 mm). Mobile phase of HNO_3 at flow-rate of 1.2 ml min^{-1}	VG PQII+. With hydraulic high pressure nebulization and desolvation system	Discontinuous elution in two steps by injection of 0.3 *M* HNO_3 for Cr (VI) and 1.0 *M* HNO_3 for Cr (III)	[149]
Lanthanides	Spallation nuclides	Irradiated tantalum target	Cation-exchange. Dionex IonPac CS5A. Various mobile phases used with gradient elution at 1 ml min^{-1}	Finnigan MAT Element Double focusing sector field. Rf 1280 W, Meinhard nebulizer and double pass spray chamber	Nucleide abundances of each lanthanide were in good agreement with theoretical values	[150]
Se	Selenomethionine, selenocystine, selenite, selenate	White clover plant sample	Anion-exchange. Merck Polyspher IC AN-2 (120×4.6 mm). Mobile phase 6 m*M* salicylate, 3% methanol, pH 8.5, with Tris. Cation-exchange. Ionosphere-C (100×3 mm). Mobile phase 2 m*M* pyridinium in 3% methanol at pH 2.9 with formic acid	Perkin-Elmer Sciex Elan 5000. Cross flow nebulizer and double pass spray chamber. ^{82}Se isotope monitored	Selenate and a compound of unknown identity were present in the plant samples	[151]
Se	Se-urea, Se (IV), Se (VI), selenomethionine Se-ethionine and Se-cystine	Bacterial material containing selenium	Anion-exchange Dionex AS11 with NaOH and TMAH as eluents. Heating the column at 30 °C improves sensitivity noticeably	Perkin-Elmer Sciex ICP-MS Elan 5000. ^{82}Se isotope monitored	LODs of 0.1 μg l^{-1} were achieved on the undiluted protease digestion (Pronase E) of the se-containing bacterial samples	[70]
Se	Selenite, Se-methionine and tri-methylselenonium	Human urine	Cation-exchange Dionex Ionpac CS5 with 30 m*M* ammonium formate buffer at pH 3 and 2% methanol	Perkin-Elmer Sciex ICP-MS Elan 6000 with glass concentric nebulizer and cyclonic spray chamber. $^{82,77}Se$ isotopes monitored	Anion-exchange solid-phase extraction of urine after whirlmixing with 50 m*M* benzo-15-crown-5-ether and centrifugation was done	[43]
Se, As, Cr	As (III), As(V), mono-methylarsenic, di-methylarsenic, Se (IV), Se (VI) and Cr (VI)	Fresh water	Anion-exchange PRP-X100 Hamilton with gradient elution of 20 m*M* and 60 m*M* NH_4NO_3 at pH 8.7	Hewlett-Packard 4500. Babington nebulizer. ^{53}Cr, ^{75}As, ^{77}Se isotopes monitored	LODs below 0.06 μg l^{-1} for As, 1.5 μg l^{-1} for Se and 0.18 μg l^{-1} for Cr	[68]

Taken in part from Sutton and Caruso [133], with permission of J. Chromatogr. A.

3.1.4. Ion-exchange chromatography

Ion chromatography has been a chromatographic method for the determination of inorganic ions. Gradually, this technique has broadened in scope to include organic ions, other separation methods (e.g., ion interaction and ion exclusion) and simultaneous separation of anions and cations. Lopez-Ruiz [34] and Buchberger [35] give excellent reviews for applications of ion chromatography.

The separation of the analytes in ion-exchange chromatography takes place when the analyte ion and the ions of the mobile phase compete for the oppositely charged functional group ions on the stationary phase. The relative affinities of the solute ions determine the extent of retention in the stationary phase. Ion exchange is not only used for the separation of inorganic ions but to any easily ionized substance [36] and it can be used in two modes: anion-exchange or cation-exchange. Although anion-exchange has been more frequently used for speciation analysis, cation-exchange has found some useful applications. Analysis of bromate in drinking water was proposed by Creed et al. [37] and later improved by Seubert and Nowak [38] allowing the determination without any sample pretreatment. Other analyses for inorganic bromide, iodide and sulfur have been developed [39–41]. Selenium determination has also been achieved comparing two ion-exchange methods [42]. More recently, selenium was speciated by ion-exchange chromatography in human urine samples [43]. Arsenic ions such as the organoarsenicals and arsenosugars have been widely studied by anion-exchange [44–64] and, in recent times, two methods were developed for the simultaneous separation of anionic and cationic arsenic species in a single injection [65]. Speciation of halides and oxyhalogens has been described for the simultaneous analysis of chloride, chlorite, chlorate, perchlorate, bromide, bromate, iodite and iodate [66]. Other applications such as speciation of arsenic in apples [67], in fresh water [68,69], or selenium in bacterial material [70] and urine samples [43] are illustrated in Table 3. Fig. 3 presents the chromatogram for the separation of arsenic species by anion-exchange with 2 m*M* NaH_2PO_4 and 0.2 m*M* EDTA at pH6 obtained by Day and co-workers [69].

3.1.5. Size-exclusion chromatography

This type of chromatography differs from those

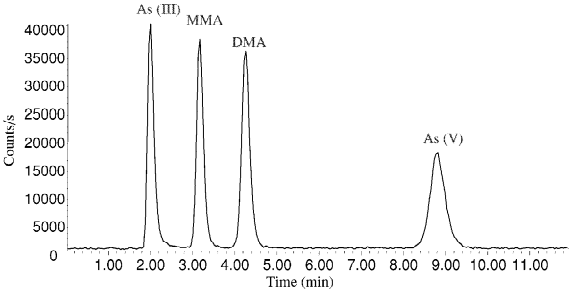

Fig. 3. Chromatogram showing the separation of 1 ppb arsenic species with 2 m*M* NaH_2PO_4 and 0.2 m*M* EDTA at pH 6 mobile phase [69].

above in the performance of the stationary phase. The stationary phase acts as a sieve to separate molecules based on their physical size. Typically the smaller molecules are held back and the larger molecules pass through. This type of separation is ideal for high-molecular mass compounds such as proteins, polymers and peptides. The stationary phase consists of a network of pores where molecules larger than the pore sizes are excluded from the pores, thus eluting rapidly. In the same manner, molecules smaller than the pores, will have more pathway options and will take longer to elute. The molecules between these two extremes are fractionated by their size. They diffuse through the pores at rates determined by their size and shape, thus eluting at different times. Since the retention time is not based in chemical interactions, the mobile phase does not play a critical role. The mobile phase must only retain the conformational structure of the molecule that resembles its native form. Typical mobile phases are buffers with neutral pH. The original stationary phase used in size exclusion was starch, which was not very durable. A new material was then used, Sephadex, which had been used in zone electrophoresis. Today, the most widely spread stationary phase for biochemical applications are different pore sizes of Sephadex or similar products, depending on the range of molecular weights to be separated. Size-exclusion chromatography can be calibrated with respect to molecular mass and therefore offers the possibility to determine the molecular masses of unknown samples according to their retention time.

Size-exclusion chromatography has grown in popularity because of its application for the analysis

of biological samples. There have been a number of applications with ICP-MS detection, since it offers the possibility of selective detection. For a number of applications this separation technique is followed by a complementary liquid chromatographic technique [71,72].

Separation of metalloproteins using SEC has been studied by several groups [73–77]. The use of SEC has become an important tool in the speciation studies of dissimilar biological samples such as human fluids [78–80] and plants [81,82]. But recently, SEC has been used in more diverse application such as iron speciation in meat [83], speciation of black tea [84] and quantification of metal organic interactions [85].

3.1.6. Chiral liquid chromatography

The growing awareness of the different properties of enantiomers in the pharmaceutical industry and the fact that the biochemistry of living organisms is enantio-selective, has spurred considerable effort to the preparation and separation of enantiomers. One compound can have several enantiomers, and their separation can be achieved either by adding a chiral additive to the mobile phase, by using a chiral stationary phase or by forming chiral derivatives. Chiral additives tend to be expensive and not readily available. Also, the presence of chiral additives in the mobile phase may interfere with detection or recovery of the analyte. The use of chiral stationary phases would seem to be the better choice of the two. Separation of selenoamino acids has been achieved with β-cyclodextrin columns followed by hydride generation before introduction into the ICP-MS [86], crown ether columns with an acidic mobile phase and direct coupling to ICP-MS [11,87] and a teicoplanin column with a neutral mobile phase and direct coupling to ICP-MS [88]. Fig. 4 shows the enantiomeric separation obtained by Ponce de Leon et al. [87] for eight different seleno amino acids made with a crown ether column and ICP-MS detection at three different temperatures.

An alternative approach is to derivatize a chiral analyte with an optically pure reagent, producing diastereomers (an analyte with no chirality), which can then be separated by conventional chromatography since the enantiomers will have been converted to entities with differing physical properties.

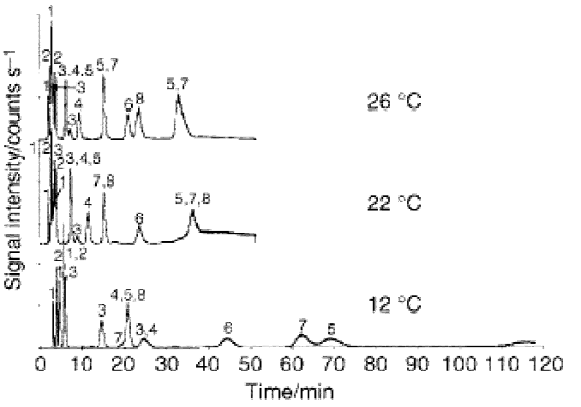

Fig. 4. Chromatogram showing the separation of eight selenoamino acids at different temperatures. (1) Se-lanthionine; (2) Se-methylselenocysteine; (3) Se-cysteine; (4) Se-cytathionine; (5) Se-adenosylselenohomocysteine; (6) γ-glutamyl Se-methylselenocysteine; (7) Se-ethionine; (8) Se-homocysteine. From Ref. [87], reproduced by permission of The Royal Society of Chemistry.

Montes et al. [89] used Marfey's reagent for the derivatization and separation of seleno amino acid enantiomers.

3.2. Gas chromatography

The methods of choice for separation of organometallic species in environmental and biological materials are determined by the physicochemical properties of the analyte (volatility, charge, polarity, molecular mass). Although HPLC separations have been widely employed in most speciation studies (especially in the biological field), gas chromatographic techniques present interesting alternatives. Flame ionization (FID), electron capture (ECD) or flame photometric detection (FPD) are well known detection systems; they have been used with packed and capillary columns for a wide variety of analytes (mainly organic or halo-organic species). In terms of elemental selective detectors, coupling of microwave-induced plasma-atomic emission spectrometry (MIP-AES) to GC was the first commercial instrument made available by Hewlett-Packard (now Agilent Technologies). The analytical capabilities exhibited by this system for selective detection of elements such as Pb or Sn in organometallic molecules and halogens as Cl or Br suggested that

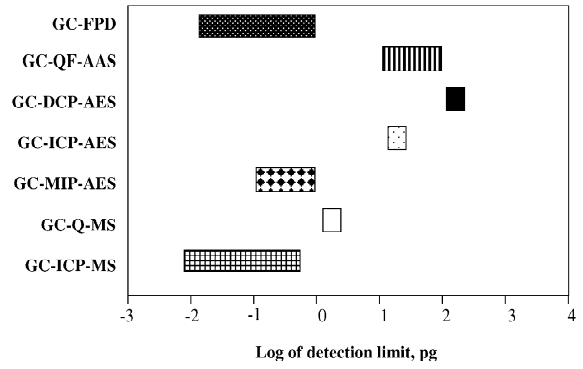

Fig. 5. Detection limits (as absolute mass in picograms) obtained by using GC as sample introduction with different detectors. Data obtained from Ref. [90].

plasmas were highly desirable as detectors for GC separations.

Fig. 5 shows the detection limits achievable using GC as the separation technique coupled to different detectors. As can be observed, the coupling to ICP-MS provides the best results in terms of detection limits and, therefore, this has become a powerful option for the speciation of the organometallic compounds. It is widely employed in environmental samples, to analyze samples, providing they are volatile enough or can be efficiently derivatized. For analytical plasmas, gaseous sample introduction is the ideal approach (no desolvation/volatilization processes are required). However, this coupling is not as straightforward as the HPLC coupling since condensation effects of the analytes have to be avoided during their transport from the GC to the ICP-MS. Also, the effluent from the gas chromatograph (a few ml per min) requires an additional carrier gas to achieve sufficient flow in the central channel of the plasma.

Since no GC–ICP-MS interface was commercially available until recently, most of the published papers devoted to the use of GC–ICP-MS required interface development. The first coupling reported between GC (packed columns) and ICP-MS was done by Chong and Houk [90], but since the introduction of capillary columns they have become the preferred option for higher sensitivity and resolution. The first publication was by Ebdon and co-workers [91]. Their interface involved using a rigid aluminum bar heated by a variable resistance voltage supply and coupled to a demountable torch. This design was evaluated for several organometallic compounds including lead, tin and mercury with satisfactory results [92]. The most challenging application was the speciation of metalloporphyrins of geological interest, which exhibit high boiling points [93].

An interface design representing an important contribution to the development of GC–ICP-MS has been the one from De Smaele et al. [94–96]. This interface has been applied for the determination of Sn, Pb and Hg mainly in environmental samples. Detection limits in the ppt range for most of the studied compounds were obtained with this device specially using solid-phase microextraction (SPME) as sample introduction technique (0.13–4 ppt as metal were reported) [97,98]. The same authors documented the continuous monitoring of Xe as internal standard to test the system for instrumental drift.

A interesting interface design has been proposed by Montes-Bayón et al. [99] using a non-heated flexible interface design. The schematic of this

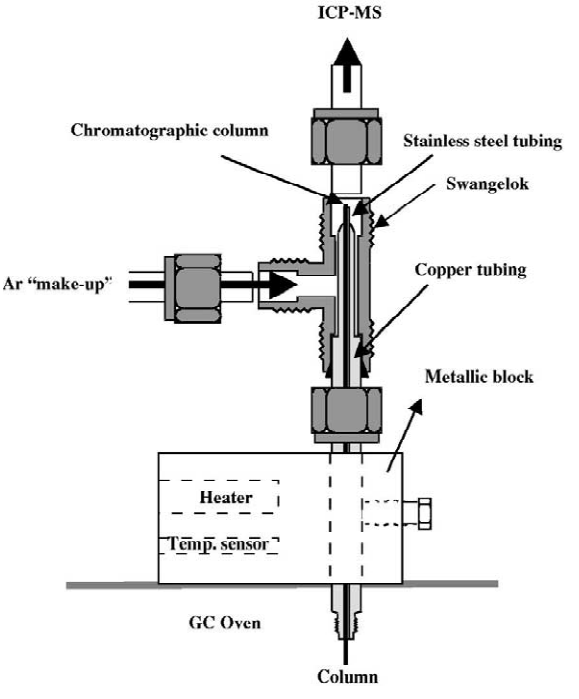

Fig. 6. Schematic of the flexible interface design obtained in Sanz-Medel's group. From Ref. [99], reproduced by permission of The Royal Society of Chemistry.

interface is shown in Fig. 6. It mainly consists of a metallic T-piece connected to a concentric assembly of copper tubing and an internal stainless steel tube where the end of the column (about 10 cm) is placed. The copper tube is inserted into a metallic block heated by an electric heater and a temperature sensor, both controlled by the GC. This interface has been evaluated for the speciation of organometallic compounds containing mercury [100], tin [101] or lead [102] but also for the separation of Se-amino acids [103] and their enantiomeric forms [104]. A primary feature of this design is the flexibility of the system that allows the free movement of the torch box, especially important during the ignition step in certain instruments. However, the non-heated approach compromises the signal for high boiling point species.

Agilent Technologies has commercialized an interface design that possesses the advantage of flexibility, but that is heated up to and into the torch. The system is also based on a demountable torch and the temperature is controlled by the GC. The papers published with this interface (prior to its commercialization) showed very promising results for the determination of organotin compounds in environmental samples (sediments and sea-water samples) [105,106]. Detection limits in the low femtogram range were reported. Fig. 7 shows the chromatogram obtained for a standard containing 10 ng ml^{-1} (as Sn) of 10 organotin compounds: monobutyl tin (MBT); tripropyl tin (TPrT); dibutyl tin (DBT); monophenyl tin (MPhT); tributyl tin (TBT); tetrabutyl tin (TeBT); tripentyl tin (TPeT); diphenyl tin (DPhT); triphenyl tin (TPhT) [105,106].

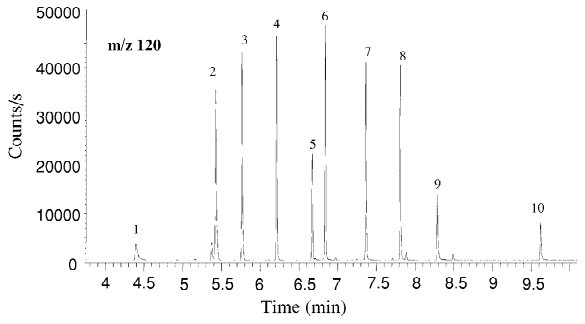

Fig. 7. Organotin compounds by GC–ICP-MS with Agilent Technology patent. One microliter injection of 10 ng ml^{-1} as Sn for every compound: (1) tetraethyl tin; (2) monobutyl tin; (3) tripropyl tin; (4) dibutyl tin; (5) momophenyl tin; (6) tributyl tin; (7) tetrabutyl tin; (8) tripentyl tin; (9) diphenyl tin; (10) triphenyl tin.

One of the novel trends in the field of coupling GC to ICP-MS for trace elements speciation is the application of isotope dilution techniques. The use of species-specific spikes (SSS) has proved suitable to improve precision and accuracy in the determination of mercury species in aquatic samples and species transformation processes [107]. The main limitation of the method is the required synthesis of the enriched $Me^{201}Hg+$ species to be spiked in the sample. With the same type of methodology, it was possible to accomplish accurate organotin species quantification in sediments by spiking isotopically enriched (^{119}Sn) monobutyl, dibutyl and tributyl tin compounds [101]. Species transformation/degradation was successfully evaluated for different extraction techniques including ultrasound or microwave extractions with a methanol–acetic acid mixture as extractant [108].

3.3. Supercritical fluid chromatography

Supercritical fluid chromatography has been referred as the missing link to unify the chromatographic techniques. SFC combines the best features of gas, i.e., the high coefficient of GC and liquid chromatography, i.e., the high solubility, making it possible to easily separate compounds which customarily are difficult to separate such as thermally labile, non-volatile and high-molecular mass compounds and/or accomplish the separation in shorter times. As its name states, the mobile phase is a gas in its supercritical state. The most common gas used as a mobile phase is carbon dioxide, although other gases such as nitrous oxide [109] have been used. Since carbon dioxide in the supercritical state exhibits a polarity similar to hexane, separation of highly polar compounds or ionic compounds becomes difficult. To overcome this problem, modifiers have been added to the carbon dioxide mobile phase. The most common modifier used for carbon dioxide are alcohols such as methanol, but other modifiers such acetic acid [110] or acetonitrile [111] have been used. Although modifiers offer options to improve the separation of the analytes, the main variable to control is pressure.

When SFC is coupled to ICP-MS, the mobile phase changes from a supercritical state to a gas, which the ICP-MS instruments can handle easily. The main problem is cooling at the end of the restrictor due to the Joules-Thompson effect. Therefore, a high temperature must be maintained to avoid cluster formation and wall condensation [112]. Micro or capillary columns are preferred when interfacing with ICP-MS, since lower flows of carbon dioxide diminish the carbon deposits on the cones.

Interfaces for SFC to ICP-MS are based on the original design by Shen et al. [113] consisting of a heated transfer line containing the restrictor, whose end is mounted in a heated copper tube. The tube is inserted into the central tube of a regular ICP-MS torch. The design is similar to the GC–ICP-MS interface except for a three-way Swagelock union used to introduce heated argon as a make-up gas at about 200 °C to avoid cooling at the restrictor tip and transport the analyte to the plasma.

Limited applications to speciation studies with SFC-ICP-MS are found in the literature, but development of new commercial instruments that can perform both LC and SFC will certainly increase its use in speciation studies including chiral separations. A recent review for SFC-ICP-MS applications by Vela et al. [114] has been published. The capabilities of this technique have been demonstrated with organometallic compounds of tin [113,115–117], lead [115,116,118], chromium [109,119], mercury [120,121] and arsenic [117,121].

3.4. Capillary electrophoresis

Although not strictly a chromatographic technique, capillary electrophoresis (CE) is a versatile separation technique easily coupled to an ICP-MS. CE has the ability to separate cationic, anionic and neutral species (as can HPLC), use extremely small sample and change column properties by simply changing to a new solution. It also has the advantage over HPLC of having fast elution times, no peak broadening since there are no mechanical pumps. CE separates the analytes using the electroosmotic flow phenomenon. In its simplest form, CE consists of applying a constant dc potential along a capillary filled with a conducting aqueous buffer solution. The analytes within the sample separate due to their differences in mass-to-charge ratio as well as the resulting electrophoretic migration rates.

Since CE utilizes very small sample volumes (1–100 nl), the detector should be a sensitive one. ICP-MS has the capabilities for handling small sample volumes with high sensitivity. Olesik et al. [122] performed the first hyphenation between CE and ICP-MS using a conventional concentric nebulizer. Since then, several modifications have been made to overcome the coupling problems for CE and ICP-MS such as the back pressure produced by the nebulizer gas when it exits the nebulizer or stable electric connections. The best approach to suction effects has been using self-aspiration of the electrolyte buffer. A complete discussion of CE and its coupling to ICP-MS is given by Olesik et al. [122]. More recently, an interface designed with a modified concentric nebulizer to minimize dead volumes, has been done by Tangen and Lund [123].

Applications of CE coupled to ICP-MS include separation of lanthanides [124], metalloporphyrins [125], chromium in water [126] and antimony speciation [127]. Metallothioneins isoforms separation seems to be particularly good with CE, as seen in Chasaigne et al. [128] as well as by Polec et al. [129]. Fig. 8 presents the electropherogram for the rabbit metallothionein isoforms with different metals.

Selenium has become of special interest due to its anticarcinogenic properties and several CE publication have appeared, Michalke [130] gives a good overview on the subject. Chiral analysis on D,L-selenomethionine has been done by Day et al. [131] using CE with UV and ICP-MS detection.

4. Conclusions

Chromatographic separations (including CE) coupled with ICP-MS for detection serve as a powerful combination for elemental speciation studies. The high separation powers of modern chromatography combined with both ppt detection levels, and the specificity of ICP-MS provide the analyst an excellent tool. The temporal separation is augmented by the excellent element specificity of the ICP-MS. Together they serve to resolve in time what cannot be spectrally resolved and vice versa.

At the time of this writing the LC techniques are

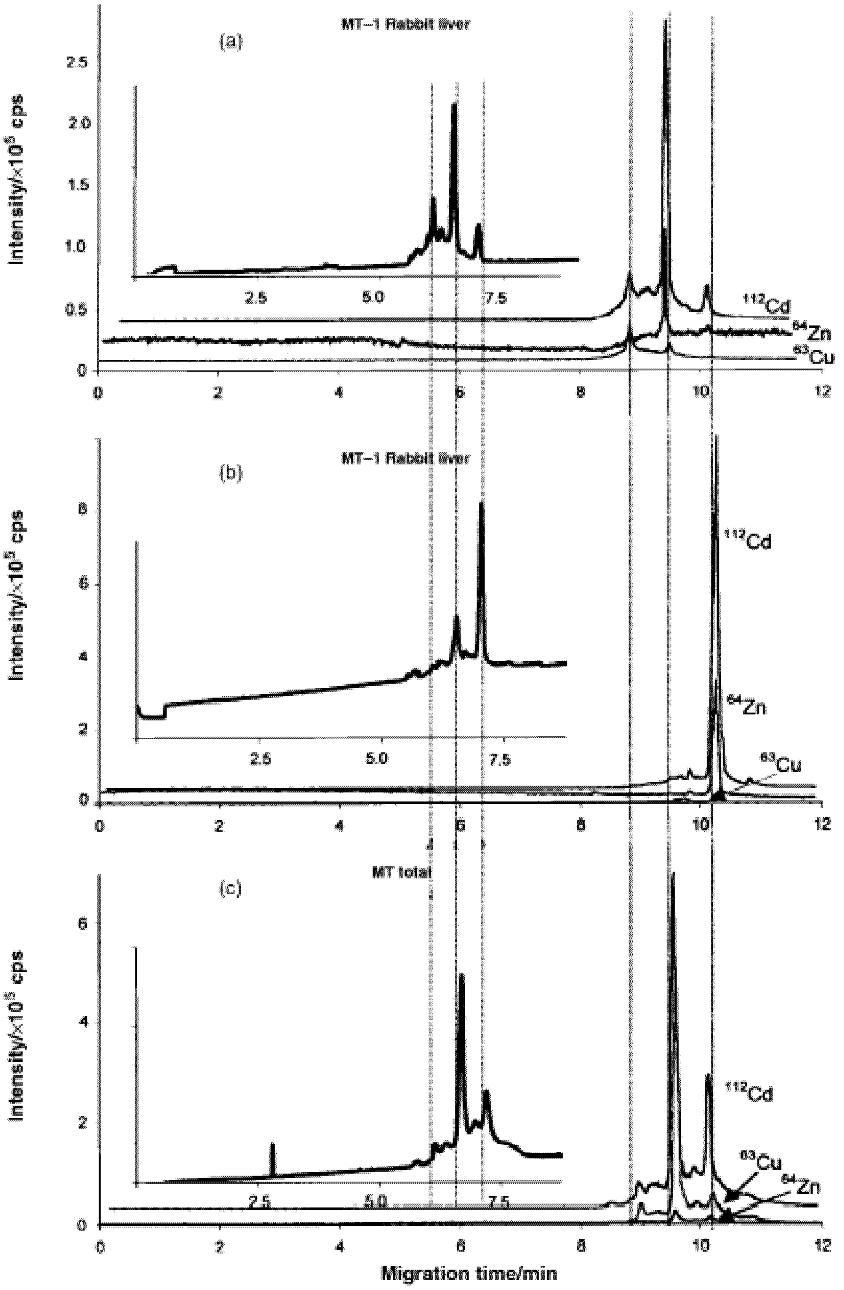

Fig. 8. Electropherogram with ICP-MS and UV (inset) detection for natural purified rabbit metallothionein (a) MT-1, (b) MT-2 and (c) MT mixture prior to separation into isoforms. From Ref [129]., Reproduced by permission of The Royal Society of Chemistry.

those most often utilized with ICP-MS detection. Of these, both ion-exchange and reversed-phase chromatographies are the most widely used. Ion-exchange methods with element-specific detection are primarily anion-exchange. These provide ready applicability to important anionic species such as those from As and Se. Reversed-phase with ion-pairing provides a level of flexibility allowing both

polar and non-polar species to be separated in one run. Sequencing to the detector is then straightforward, since the ICP makes no distinction between these types of species at low levels. Size exclusion chromatography is steadily increasing in importance with ICP-MS detection. This provides direct detection and the ability to prescreen for chromatographic techniques that will be necessary to adequately address the complex samples provided by biological and environmental samples.

GC is becoming more important for volatiles in coupling with ICP-MS. In a sense it is an ideal method for sample introduction to the ICP. It offers a gas phase sample presentation, which results in lower detection levels than those for liquid aerosol introduction because the desolvation and vaporization steps are eliminated. Therefore more plasma energy can go to ionizing the element of interest. Further, with modest sample pretreatment, such as with solid-phase micro-extractions, matrix can be eliminated and direct injection to the GC accomplished. Similarly, SFC provides a gaseous sample introduction to the ICP and is not limited to only volatiles or volatile derivatives. Unfortunately, SFC-ICP-MS has not been widely investigated even though it is a potentially powerful technique.

CE is a separation technique that has gained a modest level of popularity using ICP-MS detection and an interface is now commercially available to solve the problems of securing an electrical connection and compensating for nebulizer suction. Viable and useful electropherograms are obtained but at poorer detection levels than for GC and LC. The analyte mass in the nl injection volumes is very low, limiting the technique to date, to sub-ppm.

Sample preparation is also a key part to the success of a speciation study. Szpunar et al. [132] give an excellent overview for sample preparation for the elemental speciation studies.

Overall, elemental speciation by chromatography–ICP-MS is making good progress as an important analytical tool. Miniaturization of these systems is also rapidly developing since on-site species determination is becoming more compelling. Also, the use of complementary techniques for structure determination is becoming more widespread. One example is the use of electrospray mass spectrometry (ES-MS), which has gained acceptance due to its capability of being coupled to liquid chromatography.

Speciation versus total trace element analyses is gaining more popularity and we can expect a wider use in the future.

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