Atomic Fluorescence Spectrometry: a suitable detection technique in speciation studies for arsenic, selenium, antimony and mercury

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Atomic Fluorescence Spectrometry (AFS) is an ideal detection technique for speciation studies concerning hydride forming elements (mainly As, Se and Sb) and Hg. The analytical features of AFS, such as detection limits below the μg L^{-1} and the wide linear calibration range, up to the mg L^{-1}, allow its application to a great variety of environmental, biological and food samples. AFS represents a suitable alternative to other atomic spectrometers commonly employed in speciation studies such as Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The instrumentation used for AFS and the design of the vapour generation and optical layouts required to sustain the full benefits of the AFS approach are also described. The present review explains and comments on the instrumental couplings of chromatographic (HPLC and GC) and non-chromatographic separations (CE) with AFS detection, with online hydride generation for the speciation of inorganic and organic compounds of As, Se and Sb, and cold vapour for Hg. Other optional intermediate steps are online photo-oxidation (UV), pyrolysis or Microwave Assisted Digestion (MAD) for non-directly reducible compounds. Many different sample types (*e.g.* water, soils, air, biota, food) have been analysed using these instrumental couplings with AFS detection. These are summarised and discussed.

1. Introduction

Speciation has been defined as the distribution of an element amongst defined chemical species in a system.[1] Speciation has become a common and useful tool in several scientific fields as it provides information about the properties (*e.g.* bioavailability, mobility and toxicity) of the different chemical species in which an element can be distributed. The importance of speciation studies has resulted in an abundant scientific literature describing speciation of several metals (Hg, Se, Cr and Sn) and metalloids (As and Sb) in environmental, biological and food samples.[2,3]

Most analytical methods employed for speciation involve the hyphenation of separation techniques, mainly chromatography

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(GC and HPLC) with specific element detectors based either on atomic absorption (AAS), atomic emission (ICP-AES), or mass spectrometry (ICP-MS).[4,5] Hydride Generation (HG) for As, Se and Sb, and Cold Vapour (CV) generation for Hg are commonly used as online post-column derivatisation to separate and pre-concentrate the analytes from sample matrices, thus eliminating potential interferences in the detection that may arise in some direct couplings (*e.g.* LC-MS with an electrospray ionization interface).[6]

AFS represents a suitable alternative to the other atomic and mass spectrometric techniques. AFS has been described to be superior to AAS[7,8] and similar to ICP-MS regarding sensitivity and linear calibration range, with further advantages (simplicity, lower acquisition and running costs) for arsenic speciation[9,10] and selenium speciation[11] in routine analysis. AFS provides interesting analytical features, such as low detection limits (below the μg L^{-1}) and wide linear calibration range (from μg L^{-1} to mg L^{-1}).[12] AFS spectrometers are commonly based on the use of non-dispersive instruments, equipped with Boosted Discharge Hollow Cathode Lamps (BDHCLs) as the excitation radiation source. The volatile species of As, Se and Sb, obtained from liquid samples after HG, are carried with an argon flow to a gas–liquid separator, followed by atomization and excitation in an argon–hydrogen diffusion flame. For Hg, the CV technique is employed, with no need of a flame for atomization. For those chemical species that do not readily form volatile species, such as some complex organometallic species, additional online derivatisation steps are needed (*e.g.* photo-oxidation, pyrolysis or microwave digestion) before HG or CV.

The use of AFS as a suitable detector for speciation studies has only recently been considered. An early review on As and Se speciation by HPLC hyphenated to specific detectors[13] mentions barely 10 references based on AFS detection out of a total 152 references. The potential use of AFS in Hg, Se and As speciation studies was also briefly indicated in a review in 2000, when AFS was described as a promising possibility for speciation studies.[14] The use of AFS detection is also mentioned in a more recent review for mercury,[15] as well as another review for arsenic speciation.[16] However, in these reviews, the number of references dedicated to AFS is rather limited. A recent review on speciation based on non-chromatographic separations also includes AFS detection.[17]

The present review aims to give a critical vision of the state of the art of speciation studies of As, Se, Sb and Hg based on AFS detection, considering the extensive number of scientific articles published in the last ten years. The present review describes and comments on the instrumental couplings of chromatographic (HPLC and GC) and non-chromatographic separations (Capillary Electrophoresis, control of chemical conditions involved in HG or CV) with AFS detection. Many different sample types (*e.g.* water, soils, air, biota, food) have been analysed using these instrumental couplings with AFS detection. These are summarised and discussed. Future developments will also be considered.

2. Atomic Fluorescence Spectrometry instrumentation

Atomic fluorescence is a spectroscopic process which is based on absorption of radiation of specific wavelengths by an atomic vapour with subsequent detection of radiationally deactivated states *via* emission in a direction (typically) orthogonal to the excitation source. Both the absorption and the subsequent atomic emission processes occur at wavelengths which are characteristic of the atomic species present. AFS is a very sensitive and selective method for the determination of a number of environmentally and biomedically important elements such as mercury, arsenic, selenium, bismuth, antimony, tellurium, lead and cadmium.

The main types of atomic fluorescence are (a) resonance fluorescence, (b) direct line fluorescence and (c) stepwise line fluorescence. Resonance fluorescence occurs when atoms absorb and re-emit radiation of the same wavelength, this is the predominant form of fluorescence measured by analytical

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chemists. These wavelengths can be different. Direct line fluorescence is quenched when an atom is excited from the ground state to a higher excited electronic state and then undergoes a direct radiational transition to a metastable level above the ground state. Stepwise line fluorescence occurs when the upper energy levels of the exciting and the fluorescence line are different. The excited atoms may undergo deactivation, usually by collisions to a lower excited state rather than return directly to the ground state.

The intensity of the fluorescence radiation depends on a number of factors: (a) the intensity of the excitation source, (b) the concentrations of the atoms *i.e.* the atomiser, (c) the quantitative efficiency of the process (*i.e.* the ratio of the energy emitted in the fluorescence to the energy absorbed per unit time) and (d) the extent of any self-absorption in the atomiser. The fluorescence radiation is linearly dependent on the source radiation and the fluorescence quantum efficiency of the transition as long as saturation is avoided. If the atomic concentration is low the fluorescence signal varies linearly against the total atomic concentration. AFS intensity is additionally proportional to the concentration of analyte in the sample and the optical efficiency of the instrument industry—the solid angles used for excitation and collection of radiation.

The stated disadvantages of atomic fluorescence are (a) quenching and (b) interferences. Quenching occurs when excited atoms collide with other molecules in the atomisation sources. Those processes are discussed in more detail by several good reviews.[18,19] An additional disadvantage of "generic" AFS is source scatter and atomizer emission causing spectral interferences. These are then minimal when HG and CV are used.

As with other techniques interferences are of two major types. Spectral interferences occur when lines in the source overlap lines in the matrix elements in the atomiser. Chemical interferences result from various chemical processes during atomisation that reduce the population of free atoms.

The basic layout of an AFS instrument is similar to AAS except that the light source and detectors are placed at right angles.

Instruments for AFS can be categorized into dispersive and non-dispersive, depending on wavelength selection. Dispersive instruments require a low resolution monochromator if a line radiation source is employed, but for a continuum radiation source a high resolution monochromator is required. In non-dispersive instruments monochromators are not employed, resulting in a simpler design and lower costs. Non-dispersive instruments can be prone to interferences, due to stray light and background emission from the atomizer.[20] In non-dispersive instruments equipped with line-radiation source, these interferences can be minimized using a filter that allows a defined bandwidth to reach the detector. In most AFS systems wavelength selection is achieved using a filter located between the source and the detector.

A number of excitation sources have been used in AFS, primarily spectral line sources and continuous sources. Since the intensity of the fluorescence radiation is proportional to the exciting radiation, excitation sources with a high intensity are required in order to achieve good sensitivity and wide linear dynamic range. The source should be simple and easy to use, have good short term and long term stability and require minimum maintenance to obtain optimum performance. The BDHCL meets these requirements. This is commercially available and this has contributed significantly to the availability of commercial AFS instruments. Most atomisers used for AFS are similar to those used in AAS or AES. The basic requirements are, for an efficient and rapid production of free atoms with minimal background noise, long residence time for the analyte in the optical path and low quenching properties. In addition ease of handling and economic cost of operations are also important.

2.1. Mercury by cold vapour

Mercury has a significant vapour pressure at room temperature and it therefore can be measured without additional thermal energy. Mercury vapour produced by reduction of the metal from its compounds with a suitable reductant (sodium

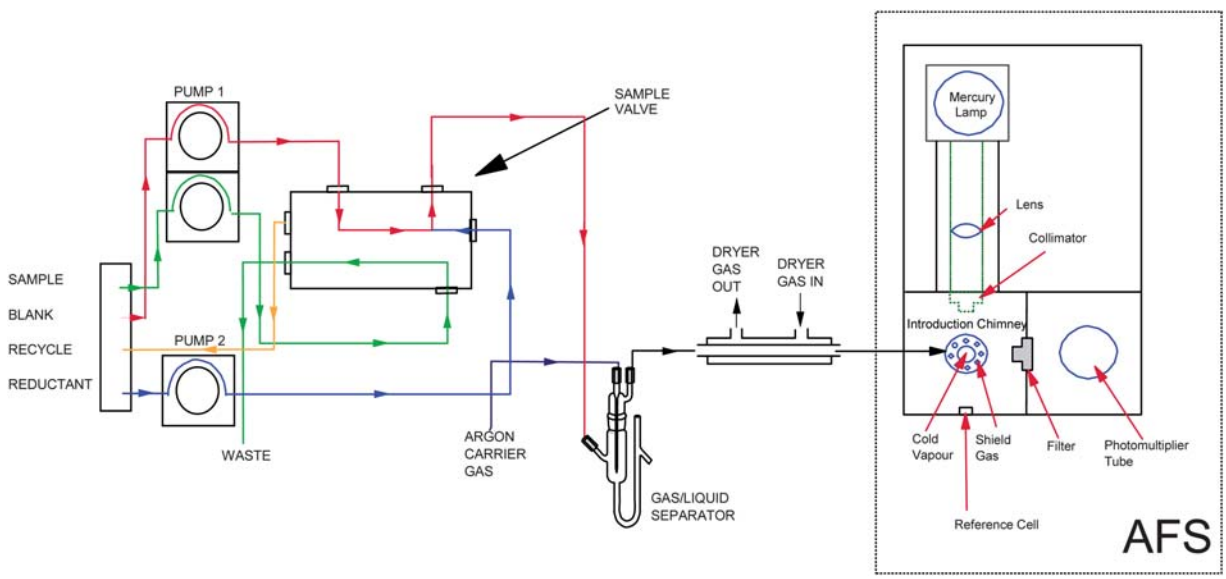

Fig. 1 Schematic diagram of the continuous flow vapour/hydride generator and optical configuration of an AFS system for mercury analysis.

borohydride or tin(II) chloride) is swept out of the reaction vessel using an argon carrier gas and introduced into the optical beam where the mercury atoms can be excited by a suitable source, *e.g.* a mercury discharge lamp. A schematic optical arrangement for mercury measurement is shown in Fig. 1. The AFS radiation is usually detected using a photo-multiplier tube (PMT). The instrument uses a ratiometric approach to compensate for lamp drift by relating the PMT signal to the reference cell signal.[21]

For any analytical procedure there are several aspects of the cycle that need to be addressed and controlled. Stockwell and Corns have discussed this in some detail with respect to automated analysis.[21] Without doubt the most important is the sampling itself so that a fully representative sample is taken which has not been compromised in any way or form.

For the determination of mercury (and also for hydride forming elements), there are two fundamental parts of the measurement cycle that must be operated in a controlled manner. These are firstly the chemical process of generating the analyte in a vapour form and secondly the transference of the vapour for quantification of the analyte using a specific AFS instrument. Attention to detail with these parameters is extremely important.

A schematic diagram of a continuous-flow vapour generator is shown in Fig. 1.[22] The reductant, blank and sample solutions are delivered by variable-speed multichannel peristaltic pumps. An electronically controlled switching valve alternates between blank and sample solutions and two of the liquid streams (reductant and sample or reductant and blank) are butt-mixed in the sample valve, where the reaction starts to occur. The streams and all gaseous products are continuously and rapidly pumped into a glass gas–liquid separator, from which the gaseous products are carried, by argon gas through a dryer system, finally reaching the AFS detector.

The design of the AFS detector for mercury analysis is relatively simple owing to the absence of a thermally energised atomiser.[23] Generally, a UV mercury vapour discharge lamp is used as excitation source and the fluorescence light is detected by a PMT, which is positioned perpendicular to the excitation source. In addition, Fig. 1 illustrates the vapour generation system and optical configuration of an AFS system.[24]

A glass chimney is used for introduction of mercury vapour into the optical path. The chimney is shielded with a high flow rate of argon. A 253.7 nm interference filter is employed between the introduction chimney and the PMT to keep stray light away from the latter.

Since the system is used to determine total inorganic mercury (Hg^{2+}), the first requirement for performing analysis is to liberate all mercury compounds from the sample matrices and then convert all organic forms of mercury to Hg^{2+} by various digestion/oxidation procedures.

For natural water sample analysis the levels of interest are relatively low and the hot oxidising method using permanganate–peroxodisulfate has been found to be unsuitable for low-level mercury determination because of the high blanks found in these reagents.[25] Bromine monochloride has been found to be an excellent oxidant and preservative for total mercury in water samples,[26] working faster and more efficiently on many organomercurials.[25,26] Hydroxylamine hydrochloride is added to destroy the excess bromine before analysis with CV-AFS. This method has often been used for converting organic mercury to Hg^{2+}.[27–32]

Once all forms of mercury have been converted to Hg^{2+}, the latter is reduced to elemental mercury (Hg^0) by using either alkaline or acidified stannous chloride.

The mercury vapour produced is carried by argon gas to the AFS instrument.

2.2. Hydride-forming elements

Several environmentally important elements such as As, Se, Bi, Sb, Te, Ge, Sn and Pb can form volatile and covalent hydrides. HG is a preferred technique when hydride-forming elements are to be analysed by atomic spectrometry.[33,34] The advantage of volatilisation as a gaseous hydride clearly lies in the separation and enrichment of the analyte element and thus in a reduction or even complete elimination of interferences. In a well designed system 100% transfer of the analyte to the detector can be achieved.

Tsujii and Kuga in 1974[35] were the first to describe hydride-generation coupled to non-dispersive AFS. They reported

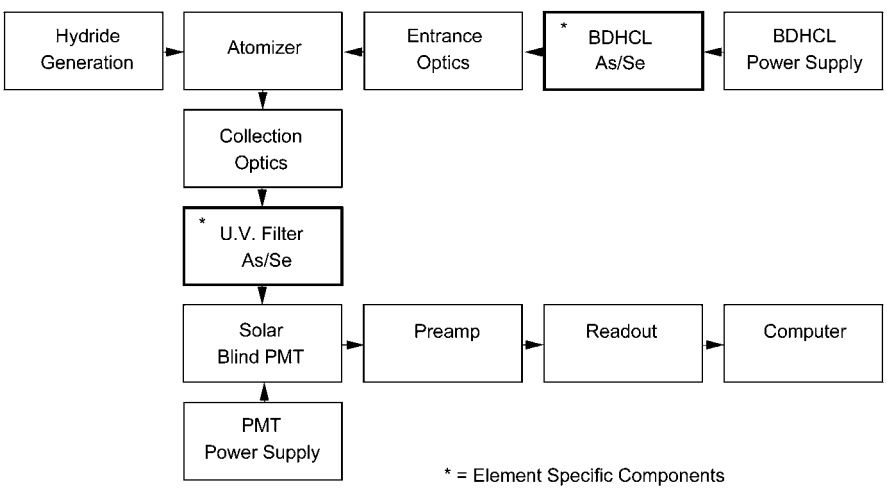

Fig. 2 Block diagram for the Excalibur Atomic Fluorescence Detector.

a detection limit of 2 ng for arsenic analysis. Thompson in 1975[36] was the first to apply a dispersive AFS system for the determination of arsenic, selenium, antimony and tellurium after hydride generation detection limits using this system ranged from 0.06 to 0.1 μg L^{-1}.

Although various metal–acid reactions (*e.g.* Zn–HCI) have been used as a means of producing hydride, $NaBH_4$ is preferred as the reductant because the technique is easy to automate, since only solutions are involved and therefore a high sample throughput can be achieved. The design of this system is similar to that of the cold vapour generation system used in CV-AFS for mercury analysis.

The schematic layout of the automated instrument to determine the hydride forming elements is shown in Fig. 2. The optical configuration is similar in concept to that used for mercury analysis but makes use of a unique multireflectance filter (MRF) to concentrate the wavelengths of interest onto the PMT detector, lowering the detection limits. The MRF is configured so that the specific fluorescence waste lighting for the element water test is collected, forming an additional effect and also, commercially available BDHCL lamps exist for all of the elements discussed.

Once the hydride has been formed and driven out of the solution, it can be directly delivered by an inert gas such as argon to the atomiser where it is excited by a fluorescence light source and measured by a detector such as solar blind PMT. Corns *et al.*[37] have investigated different types of diffusion flame atomiser for hydride forming elements. From the study they concluded that the AFS signals were optimal using an argon hydrogen flame. The atomisation in the flame is not due to thermal decomposition but due to free radicals in the flame.

As with any other technique, HG-AFS suffers from interferences. Welz[38] gave an extensive discussion about various interferences encountered in hydride generation. However, experience over a number of years has shown that with the greater sensitivity of AFS, dilution of the samples can significantly reduce or eliminate interferences. Spectral interference is not a problem for HG-AFS because the analyte element passes into the atomiser as gaseous hydride, while concomitants normally remain in the reaction vessel.

Since this review is primarily related to speciation studies, separation techniques such as HPLC and GC are used to separate the species prior to measurement. As the species are eluted they must be converted to the elemental form and care in this step is vitally important in order to maintain the integrity of the species and allow reliable measurement.

Several of the cited references refer to instrumentation that has been built in research departments, whilst others have coupled commercially available AFS instruments to commercial chromatographic systems. To the authors' knowledge, AFS instruments for the analysis of hydride-forming elements and mercury are currently being manufactured by several companies: P S Analytical Ltd, Orpington, Kent, UK, Brooks Rand Ltd, Seattle, WA, USA, Tekran Inc, Toronto, Canada and also by these companies in China: Beijing Titan Instrument Co, Beijing Rayleigh Analytical Instrument Corporation, East and West Analytical Instruments, Beijing and Beijing Kechuang Haiguang Instrument Co Ltd.

3. Speciation of As based on AFS detection

The speciation of arsenic involves several chemical species, both inorganic and organic ones. The oxyanions, arsenite and arsenate, the monomethyl and dimethyl arsenic species (MMA and DMA) generate volatile arsines when reduced with $NaBH_4$ in acidic media. There are other methylated species, such as the trimethyl arsenic oxide (TMAO), tetramethyl arsenic ion (TETRA), arsenobetaine (AB), arsenocholine (AC) and several arsenoribosides (commonly referred to as arsenosugars), that do not readily form volatile species. In this case, the destruction of the organic part of the molecules is required prior to the HG step, and can be achieved by means of either photo-oxidation (with a strong oxidant $K_2S_2O_8$ in basic media and UV radiation), thermooxidation (with a strong oxidant $K_2S_2O_8$ in basic media and heating), or a microwave (MW) assisted digestion.

The addition of an H_2 flow at the gas–liquid separator has been proposed, in order to maintain a steady flame in the AFS atomizer, and not to depend on the H_2 produced during the hydride generation step.[7]

3.1. Speciation with chromatographic separation

HPLC based on ion-exchange is the preferred separation technique for arsenic speciation combined with AFS detection, as most of the arsenic species exist in solution as negatively or positively charge species, depending on the pH. They are separated commonly by HPLC using either strong ion exchange columns[39] or in some cases with reversed phased columns with ion-paring reagents.[40,41] The use of ion exchange columns is widely employed in most speciation studies, as the separation process is more reproducible and less prone to sample matrix interferences than with reversed phased columns with ion-paring reagents. Up to twelve arsenic species have been determined in a single run with an anion-exchange column.[42] Detection limits in the μg L^{-1} level or lower are easily achieved with HPLC-HG-AFS. As an example, detection limits of 0.2, 0.1, 0.2, 0.2 and 0.3 μg L^{-1} have been reported for each of the following species: As(III), As(V), DMA, MMA and AsB (the later with a post-column UV photo-oxidation step).[12] Improvement of the detection limits has been reported with new gas–liquid separator designs.[43,44]

The HPLC-HG-AFS have been successfully applied to a great variety of samples, as summarised in Table 1. There are applications to various types of aqueous samples, such as pore water,[45] leachates from chromate-copper-treated wood,[46] wastewater,[47] gold mine tailings,[48] acid mine drainage[49–51] or beer.[52] For urine samples,[53] some authors have indicated the benefits of AFS compared to AAS[8] and ICP-MS[10] applied to speciation of inorganic species.

For inorganic solid samples, the arsenic species have to be previously extracted before injection onto the HPLC column. Both phosphoric acid and ascorbic acid solutions have been applied for extraction of polluted mining soil[54,55] or reference materials (river sediment, agricultural soil, and sewage sludge).[56,57] The extraction yield is improved by shaking, ultrasonic and microwave assisted extraction.[58] Microwave irradiation works well for the extraction of soil, sludge and related samples' extraction, with efficiencies over 90% and short

Table 1 Selected references (in chronological order) for As speciation with AFS detection

As species	Sample matrix	Instrumental coupling	Analytical features	Reference (year)
As(III), As(V), MMA, DMA, AsB, AsC, TMAO	Seaweeds, urine	HPLC-MW[a]-HG-AFS	Reversed-phase column, mobile phase: ion-pairing reagents + MeOH, sample extraction: MeOH/H_2O mixture	40 (1996)
As(III), As(V), MMA, DMA	Water	HPLC-HG-AFS	Reversed-phase column, mobile phase: aqueous TFA[b] + MeOH	41 (1996)
As(III), As(V), MMA, DMA	Urine	HPLC-HG-AFS	Reversed-phase column, mobile phase: ion-pairing reagents + MeOH	53 (1998)
As(III), As(V), MMA, DMA, AsB	Urine	HPLC-UV[c]-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer, pH 5.8	12 (1998)
As(III), As(V), MMA, DMA, AsB, TMAO, AsC, TETRA	Marine reference material	HPLC-UV-HG-AFS	Polymeric cation-exchange column, mobile phase: pyridine + citric acid, pH 2.65 polymeric anion-exchange column, mobile phase: phosphate buffer, pH 6.0, sample extraction: MeOH/water mixture	67 (1999)
AsB, AsC, TMAO	Seafood	HPLC-TO[d]-HG-AFS	Polymeric cation-exchange column, mobile phase: phosphate buffer + H_2O, pH 4.5, sample extraction: MeOH/H_2O mixture	70 (2000)
As(III), As(V), MMA, DMA	Fresh water	HPLC-HG-AFS	Polymeric cation-exchange column, mobile phase: phosphate buffer + H_2O, pH 6.0	43 (2001)
As(III), As(V), MMA, DMA, arsenosugars	Oysters	HPLC-UV-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer, pH 5.8, sample extraction: MeOH/H_2O	6 (2002)
As(III), As(V), MMA, DMA	Leachate from treated wood	HPLC-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer, pH 5.81, sample extraction: EPA's SPLP and TCLP[e]	46 (2004)
As(III), As(V), MMA, DMA, AsB, AsC, arsenosugars	Seafood	HPLC-UV-HG-AFS	Polymeric cation-exchange column, mobile phase: pyridine + HCl, pH 2.65, polymeric cation-exchange column, mobile phase: phosphate buffer, pH 5.6	71 (2005)
As(III), As(V), MMA, DMA, AsB, Nitarsone	Chicken meat	HPLC-UV-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer, pH 5.8, sample extraction: MeOH/H_2O	50 (2006)
As(III), As(V)	Acid mine drainage	HPLC-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer, pH 5.8	49 (2006)
As(III), As(V)	Mining polluted soils	HPLC-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer, pH 5.8, sample extraction: H_3PO_4 and ascorbic acids	54 (2007)
As(III), As(V), MMA, DMA	Underground water, urine	HPLC-HG-AFS	Column: polymeric anion-exchange, mobile phase: phosphate buffer, pH 6	44 (2007)
As(III), As(V), MMA, DMA	Gold mine tailings	HPLC-HG-AFS	Polymeric anion-exchange column, mobile phase: ammonium phosphate, pH 6	48 (2008)

[a] Microwave assisted digestion. [b] Trichloroacetic acid. [c] Ultraviolet photo-oxidation. [d] Thermo-oxidation. [e] Environmental Protection Agency's Synthetic Precipitation Leaching Procedure and Toxicity Characteristic Leaching Procedure.

extraction times ranging 10–20 min. Lower recoveries are found when the extraction is performed with sonication. Hydroxylammonium hydrochloride solutions have also been employed for quantitative extraction of inorganic arsenic species from atmospheric particulate matter, such as total suspended particles,[59] PM10 particles[60] and PM2.5 particles.[61] In this case, shorter extraction times (4 min) are obtained again with microwave irradiation in comparison with sonication (30 min). Other extractants such as water, $CaCl_2$ and phosphoric acid solutions have been also employed with coarse and fine atmospheric particles.[62,63]

Mild extractants, mainly methanol/water mixtures, have been reported for biological samples: seaweeds,[40] rice straw,[64] feed,[65] chicken meat,[66] marine reference materials,[67] oysters,[68] fresh

water fish[69] and seafood.[70,71] Photo-oxidation is required to determine the most common arsenic species found in biological samples (*e.g.* AsB, AsC and arsenosugars), as they do not generate volatile hydrides. Photo-oxidation to convert these species into inorganic arsenic is achieved after chromatographic separation by use of a strong oxidant ($K_2S_2O_8$) and UV light emitted from a low pressure Hg lamp. The extraction times using microwave irradiation are similar to the ones found for inorganic samples, usually ranging from 10–30 min. The combination of microwave irradiation with an ultrasonic probe allows the extraction time for chicken meat samples to be reduced to 7 minutes. The extraction time has been further reduced to 3 min using pressurised liquid extraction for biological marine samples.[72]

3.2. Speciation of As without chromatographic separation

Speciation without chromatographic separation of the analytes can be performed by adjusting the reagents ($NaBH_4$ and HCl solutions) employed for Hydride Generation. This allows the operator to select which species is converted into a volatile hydride. This off-line approach is reserved exclusively for inorganic and methylated arsenic species (As(III), As(V), MMA, and DMA). Some authors have described speciation of As(III) and As(V) in water by control of the acidity of the HG step. High acidity allows simultaneous determination of As(III) and As(V), whereas low acidity allows only As(III), as As(V) is not converted into a hydride at neutral or basic pH. As(V) is measured by difference of the two measurements. This approach has been applied to water[73] and medicines.[74] A more complete approach allows speciation of As(III), As(V), DMA, and MMA in wine, by control of the reaction media: citrate buffer for As(III), acetic acid for As(III) + DMA, 6 M HCl for As(III) + As(V). MMA is calculated by difference.[75] A similar approach is employed by other authors for speciation of the same four arsenic species in vegetables.[76]

Another non-chromatographic approach for speciation of As(III) and As(V) in water considers Multisyringe Flow Injection Analysis (MSFIA) with one multi-port selection valve. KI is needed to reduce As(V) to As(III), so As(V) is measured by difference with total inorganic As.[77]

A recent alternative to chromatographic separation is the online separation of the arsenic species by Capillary Electrophoresis (CE). CE-HG-AFS has been reported for speciation of As(III), As(V), DMA and MMA. In this case, online preconcentration (enrichment factor 37–50 fold) of the arsenic species is achieved, setting a high pH of the sample solution and low pH of the CE buffer, allowing detection limits in the range 5.0–9.3 μg L^{-1}.[78] The design of an efficient interface, gas–liquid separator, AFS atomizer, and elimination of backpressure of CE separation has also been described.[79] A microchip CE-HG-AFS version has also been described with As(III) and As(V) in water samples.[80]

4. Speciation of Se based on AFS detection

The majority of selenium speciation studies deal with two inorganic species (oxyanions selenite Se(IV) and selenate Se(VI)) and some selenoaminoacids (selecystine (SeCys), selenomethionine (SeMet), selenocisteine (SeCys(2)), selenoethionine(SeEt) and selenomethylselenocystine (SeMeSeCys)). Fewer studies also consider volatile selenium species (dimethyl selenide (DMSe), diethyl selenide (DEDSe) and dimethyl diselenide (DMDSe)). Of the two inorganic species and the selenoaminoacids, only inorganic Se(IV) forms a volatile hydride (SeH_4) during the HG step. Se(VI) has to be reduced to Se(IV) before it reaches the AFS detector. The same procedure has to be followed for the selenoaminoacids, which includes an oxidation of the organic part of the molecules. Almost all publications describe the separation of the Se(IV), Se(VI) and selenoaminoacids by HPLC, either with reverse-phase columns, anion-exchange columns, or both types combined.[81] Other alternatives to the chromatographic separation are CE and pervaporation (PV), although their use is not widely extended.

4.1. Speciation of Se with chromatographic separation

The early articles devoted to Se speciation with AFS detection determined Se(IV) and Se(VI) in aqueous media using a C18 silica column modified with didodecyldimethylammonium bromide (DDAB). The authors did not employ an intermediate HG step. Instead, they employed an ultrasonic nebulizer as interface between HPLC and AFS, which resulted in detection limits of 8.6 and 30 μg L^{-1} for the two oxyanions.[82] For the separation of selenoaminoacids, the same research group tested also a hydraulic high pressure nebulizer as interface, with detection limits of 50, 42, and 71 μg L^{-1} for SeCys, SeMet and SeEt, respectively.[83] When applied to real sample analysis (edible mushrooms), the use of hydraulic high pressure nebulisation resulted in complicated background effects and matrix problems.[84]

The first description of HPLC-HG-AFS for the Se speciation (Se(VI), Se(VI) and SeCys) employed a strong anion exchange column for chromatographic separation.[85] The reduction of the oxidation state and the destruction of the organic portion of the selenoaminoacids were performed with MW assisted digestion and online addition of a redox mixture (HCl solution of KBr and $KBrO_3$). This allowed detection limits of 0.2, 0.3 and 0.5 μg L^{-1} for selenite, selenocystine and selenate, respectively. The comparison of HG to ultrasonic nebulisation and hydraulic high pressure nebulisation corroborates the better detection limits obtained by HG.[86] HPLC-MW-HG-AFS has been used employed for speciation of D and L enantiomers of Se in breast and formula milk.[11] Online reduction after HPLC separation can also be accomplished by adding an HBr solution, heating at 100 °C. It has been applied to the determination of Se(IV), Se(VI) and SeCN in petroleum refinery wastewater and gold mine wastewater.[87]

Combinations of HPLC columns are frequently employed in Se speciation studies. Column switching with reversed and anion exchange columns has been proposed with HPLC-MW-AFS for the determination of Se(IV), Se(VI), SeCys, SeMet and SeEt in aqueous solution[88] and in Se spiked yeast, after pressurised liquid extraction (PLE).[89] A similar combination of columns has been employed for the determination of selenium species in infant formulae and diet supplements.[90] Other approaches combine both anion and cation-exchange columns.[91]

The use of UV irradiation (HPLC-UV-HG-AFS) has been also employed for the determination of selenoaminoacids in an

Table 2 Selected references (in chronological order) for Se speciation with AFS detection

Se species	Sample matrix	Instrumental coupling	Analytical features	Reference (year)
Se(IV), Se(VI)	Aqueous standards	HPLC-USN[a]-AFS	Reversed-phase column modified with DDAB,[b] mobile phase: phosphate buffer + MeOH + DDAB, pH 6	82 (1999)
SeMet, SeEt, SeCys	Aqueous standards	HPLC-HHPN[c]-AFS	Reversed-phase column, mobile phase aqueous TFA + MeOH	83 (1999)
Se(IV), Se(VI), SeCys	Spiked water samples	HPLC-MW[d]-HG-AFS	Polymeric anion exchange column, mobile phase: KAc + K_2SO_4 pH 6.5	85 (1999)
S(IV), Se(VI), SeCys, SeMet	Water certified reference material	HPLC-UV[e]-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer	92 (2000)
Se(IV), Se(VI), SeCN	Gold mine wastewater	HPLC-HG-AFS	Polymeric anion-exchange column, mobile phase: gradient elution with NaOH	87 (2001)
Se(IV), SeCys, SeMet, SeEt	Spiked food supplements	HPLC-HG-AFS	Reversed-phase column modified with DDAB,[d] mobile phase: gradient elution with ammonium acetate buffer + MeOH + DDAB	93 (2001)
Se(IV), Se(VI), SeCys, SeMet, SeEt	Water, urine	HPLC-UV-HG-AFS	Reversed-phase column modified with DDAB,[d] mobile phase: ammonium acetate buffer + MeOH + DDAB	94 (2001)
Total Se, SeMet	Brazil nuts	HPLC-UV-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer, pH 6, reversed-phase column modified with DDAB, mobile phase: ammonium acetate buffer + MeOH+ DDAB	96 (2003)
D-SeCys, L-SeMet isomers	Yeast	HPLC-MW-HG-AFS	Column switching: polymeric anion-exchange column + reversed phase column, mobile phase: H_2O + KAc solution	89 (2004)
Se (IV), SeCys, SeMet	Water, oysters	HPLC-UV-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer, sample extraction: enzymatic digestion	97 (2005)
Se(IV), SeMeCys, SeCys, SeMet	Yeast tablet, urine	HPLC-UV-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer	95 (2006)
Se(IV), Se(VI), SeCys(2), SeMet	Cow milk	HPLC-UV-HG-AFS	Reversed-phase column, mobile phase: ion-pairing TEABr	100 (2007)
Total Se, SeMet	Sesame seeds	HPLC-UV-HG-AFS	Polymeric anion-exchange column, mobile phase: phosphate buffer, polymeric cation-exchange column, mobile phase: pyridinium formate, sample extraction: enzymatic digestion	99 (2007)
Se(IV), Se(VI), SeMet, SeCys(2) SeMeSeCys	Plant leaves	HPLC-UV-HG-AFS	Polymeric anion exchange column, mobile phase: phosphate buffer, pH 6, cation exchange column, mobile phase: pyridine, pH 1.5, sample extraction: enzymatic digestion	91 (2008)

[a] Ultrasonic nebulization. [b] Didodecyldimethylammonium bromide. [c] Hydraulic high pressure nebulization. [d] Microwave induced reduction. [e] Ultraviolet photo-oxidation.

instrumental coupling similar to the one used for arsenic speciation, either with an anion exchange column[92] or a reversed-phase column modified with DDAB (didocecyldimethylammonium bromide).[93] HPLC-UV-HG-AFS has been for Se speciation in aqueous samples (water and urine)[94,95] and to a great variety of biological samples: reference material prepared from Brazil nuts,[96] oyster extracts,[97] selenium enriched pumpkin seeds,[98] sesame seeds,[99] selenious yeast tablets and spiked urine,[95] cow milk after supplementation of feeding with Se forms,[100] plant leaves[101] and edible leaves.[91] The extraction of the seleno-compounds from these biological matrices is commonly accomplished by enzymatic or basic hydrolysis, as it has been reviewed.[81] A summary of Se speciation applications based on AFS is described in Table 2.

It is worth mentioning the alternative to HG proposed by some authors,[102] based on online UV photolysis and a UV/TiO_2

photocatalyst reduction device, as the interface between HPLC and AFS. It has been successfully applied to the speciation of Se in water-soluble extracts of garlic shoots.

4.2. Speciation without chromatographic separation

Se speciation based on AFS detection without previous chromatographic separation is scarce, and most of them consider only Se(IV) and Se(VI). Flow systems combined with HG-AFS are the most common non-chromatographic approaches. The analysis of samples with and without a pre-reduction step using KBr allows the determination of Se(IV) or total inorganic selenium in milk, Se(VI) being calculated by difference.[103] FIA-HG-AFS has been described for inorganic selenium speciation in sewage and sludge samples, combining extraction and reduction in a microwave oven. This setup allows determination of Se(IV) and total inorganic selenium.[104] Also, there is an FIA application that uses online reduction with a novel electrochemical HG process for the determination of Se(IV) and Se(VI), consisting of an electromagnetic induction oven for reduction and a homemade tubular electrolytic cell as hydride generator. This alternative provides similar detection limits to conventional HG.[105]

Also, Capillary Electrophoresis has been coupled online to HG-AFS for speciation of Se(IV) and Se(VI) in water samples, using HCl for Se(VI) reduction. The detection limits are 33 and 25 $\mu g\ L^{-1}$.[106]

A recent non-chromatographic speciation study[107] considers a UV photochemical vapour generator for speciation of Se(IV) and Se(VI) with AFS detection, based on the reaction of the analyte with an organic acid under different reaction conditions. This approach gives good detection limits of 0.02 and 0.1 $\mu g\ L^{-1}$ for Se(IV) and Se(VI), respectively.

There is one reference to PV coupled to AFS, for the determination of volatile methylated Se species (DMSE and DMDSe) for slurry sampling,[108] although it presents the main drawback of pervaporation efficiencies ranging from 55–85%.

Proteomics, a particular case of speciation study, usually employs off-line non-chromatographic separations in combination with atomic detectors. Off-line separation by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) followed by HG-AFS has been reported for the speciation study of Se-containing proteins.[109]

5. Speciation of Sb based on AFS detection

There are only three antimony species that are considered in speciation studies: the two oxyanions, antimonite Sb(III) and antimonate Sb(V), and the trimethylantimony ion (Me_3Sb). All three generate volatile hydrides in acid media with $NaBH_4$ solutions, although the poorer signal for Me_3Sb can be enhanced by including a photo-oxidation step.

5.1. Speciation with chromatographic separation

Liquid chromatography is always employed as the separation technique, and most of the applications correspond to water samples. The earliest work utilizes HPLC-HG-AFS for speciation of Sb(III) and Sb(V) in water samples using a miniaturized polymeric anion-exchange column. The separation of the Sb species took place under isocratic conditions with an ammonium

Table 3 Selected references (in chronological order) for Sb speciation with AFS detection

Se species	Sample matrix	Instrumental coupling	Analytical features	Reference (year)
Sb(III), Sb(V)	Water	HPLC-HG-AFS	Polymeric anion-exchange column, mobile phase: ammonium tartrate pH 6.9	110 (2000)
Sb(III), Sb(V), Me_3Sb	Water	HPLC-HG-AFS	Polymeric anion-exchange column, mobile phase: KOH and ammonium tartrate gradient	112 (2001)
Sb(III), Sb(V), Me_3Sb	Water	HPLC-HG-AFS	Polymeric anion-exchange column, mobile phase: KOH and ammonium tartrate gradient	111 (2002)
Sb(III), Sb(V), Me_3Sb	Water	HPLC-HG-AFS	Polymeric anion-exchange column, mobile phase: KOH and ammonium tartrate gradient	114 (2004)
Sb(III), Sb(V), Me_3Sb	Sediment reference material	HPLC-UV-HG-AFS	Polymeric anion-exchange column, mobile phase: EDTA + hydrogen phthalate, sample extraction: citric and ascorbic acid	117 (2005)
Sb(III), Sb(V), Me_3Sb	Terrestrial plants	HPLC-UV-HG-AFS	Polymeric anion-exchange column, mobile phase: KOH and ammonium tartrate gradient, sample extraction: citric acid	119 (2006)
Sb(III), Sb(V), Me_3Sb	Water, soil reference material	HPLC-HG-AFS	Polymeric cation-exchange column, mobile phase: 20 mM EDTA, 8 mM hydrogen phthalate, 1 mM carbonate, pH 10	113 (2006)
Sb(III), Sb(V), Me_3Sb	Marine algae, molluscs	HPLC-UV-HG-AFS	Polymeric anion-exchange column, mobile phase: EDTA + hydrogen phthalate, sample extraction: H_2O, MeOH, EDTA, citric acid	118 (2007)

tartrate solution. Detection limits for Sb(III) and Sb(V) were 0.8 and 1.9 μg L^{-1}, respectively.[110] The same research group reported later a similar instrumental coupling for speciation of Sb(III), Sb(V) and Me_3Sb. Chromatographic separation of the three compounds was achieved using a longer column, and a gradient programme with KOH and ammonium tartrate. Improved detection limits of 0.04, 0.09 and 0.26 μg L^{-1} were obtained.[111] Other authors have also reported the speciation of the Sb(V) and Me_3Sb species in water samples and environmental samples with a similar HPLC-HG-AFS coupling.[112,113]

The difficulties in obtaining reproducible chromatograms for Sb species have been emphasized.[114] The concentration of the tartrate solution commonly used as mobile phase in gradient elution programmes is critical to avoid significant Sb(III) oxidation. Also, equilibrium time of the column was found to be critical to avoid Sb(III) double peak formation. These authors have applied HPLC-HG-AFS for speciation of Sb in terrestrial plants, using citric acid as extractant and sonication,[115] and in coal fly ash, after extraction with citrate at pH 5.[116]

As mentioned above, the introduction of a UV photo-oxidation step has been proposed in order to improve the signal of the Me_3Sb at the AFS detector. HPLC-UV-HG-AFS has been applied to the extraction of Sb species with citric and ascorbic acids in marine sediment extracts[117] and marine biota (algae and mollusc).[118] The optimization of the irradiation time and addition of $K_2S_2O_8$ solution result in a detection limit for Me_3Sb similar to that for inorganic species: 0.03, 0.04, and 0.03 μg L^{-1} for Sb(III), Sb(V) and Me_3Sb, respectively.[119] The main applications of Sb speciation with AFS detection are summarised in Table 3.

5.2. Speciation without chromatographic separation

There are few examples of non-chromatographic Sb speciation. Speciation of Sb(III) and Sb(V) is achieved in water samples by HG-AFS considering that adding 8-hydroxyquinoline effectively masks hydride generation of Sb(V), thus preventing its AFS detection. Therefore, in two independent analyses, Sb(III) or total inorganic antimony is determined, and Sb(V) is calculated by difference.[120] Also, flow injection with online preconcentration has been applied for Sb(III) and Sb(V) speciation in natural waters. Sb(III) is retained as a complex with ammonium pyrrolidine dithiocarbamate (APDC) at pH 1 in a knotted reactor, whereas Sb(V) is not. Afterwards, Sb(III) is eluted with HCl 1.5 M.[121]

6. Speciation of Hg based on AFS detection

Hg speciation considers inorganic Hg(II), alkylated compounds (MeHg and EtHg) and arylated compounds (PhHg). The separation of the mercury species can be achieved either by GC or HPLC, although GC is preferred. CV is employed with liquid chromatography as online post-column derivatisation to convert inorganic Hg to its elemental state Hg^0, using either $SnCl_2$ or $NaBH_4$ solutions. For methylated compounds additional steps are necessary, such as pyrolysis with GC separation, oxidation with either UV or an oxidising solution, prior to vapour generation AFS detection.

6.1. Speciation with chromatographic separation

When liquid chromatography is employed in combination with AFS detection (HPLC-CV-AFS), it is compulsory to include an online intermediate step in order to convert the organic mercury species into inorganic mercury. This oxidation has been achieved by various means: $K_2S_2O_8$ + Cu(II) solution for MeHg and EtHg,[122] ultraviolet radiation for MeHg[123] or addition of a KBr/$KBrO_3$ mixture in HCl.[124] The chromatographic separation of Hg species is always achieved with reversed-phase columns[124–126] with mixtures of water, organic solvents (*e.g.* MeOH) and sometimes the addition of a complexing agent (*e.g.* 2-mercaptoethanol).[125] Detection limits of 0.2 μg L^{-1} for MeHg, 0.07 μg L^{-1} for Hg(II), 0.06 μg L^{-1} for PhHg, and 0.12 μg L^{-1} for EtHg have been reported.

HPLC-CV-AFS has been applied to liquid samples (river water)[126] and solid samples. In this latter case, an extraction medium is required: 6 M HCl + 0.1 M NaCl for fish samples,[125]

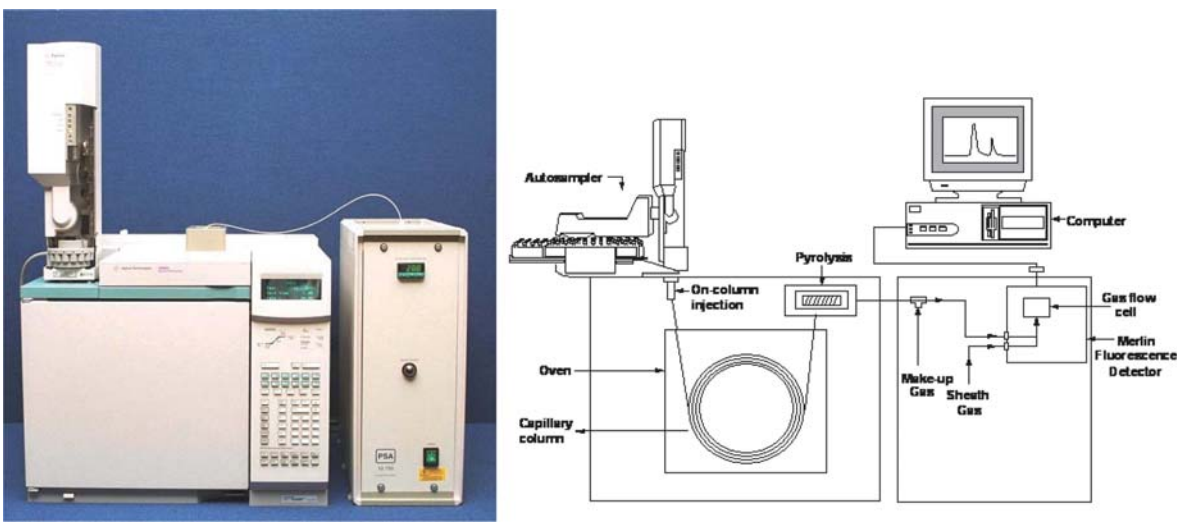

Fig. 3 Schematic diagram and photograph of the GC-AFS system.

50% aqueous MeOH + 0.2 M citric acid for marine biota (zoobenthos)[126] and river sediments.[122,123]

Gas chromatography has been more widely employed than liquid chromatography for Hg speciation with AFS detection. Although there are some early references that used GC-AFS,[127,128] most of them include an online intermediate step for Hg species oxidation, pyrolysis (pyro) being the most widely employed.[129] Before analysis by GC-pyro-AFS, all samples have to be extracted with an appropriated organic solvent. Stockwell and Lean[130] have coupled commercially available gas chromatography instruments with a specific commercial mercury atomic fluorescence detector. Fully automated instruments are therefore available and an example is shown in Fig. 3. The various mercury species are separated in the GC column and as they emerge from the outlet are pyrolysed to form mercury which is quantified on the AFS detector. Lean and Stockwell[131] have used similar systems to provide a valuable speciation service for a range of sample types. It is also a common procedure to derivatise the analytes to increase their volatility. Hg and MeHg in water samples have been extracted with dithiozone into an organic solvent and ethylated with sodium tetraethyl borate ($NaBEt_4$).[132] Ethylation has also been applied to seafood,[133] sediments,[134] biological samples and vaccines.[135] Phenylation has also been proposed for derivatisation of MeHg in marine fish products, followed by solid phase microextraction.[136,137] Enzymolysis or alkaline extraction has been employed for organomercury in food samples[138] and fish reference material.[139] Table 4 contains several applications of AFS to Hg speciation.

Detection limits of GC-pyro-AFS have been improved by some authors following different procedures. Derivatisation followed by cryogenic trapping has been successfully applied prior to GC-pyro-AFS for the determination of Hg(II) and MeHg in surface water[140] and sediments[134] with improved detection limits of 0.13 ng L^{-1} for Hg(II) and 0.01 ng L^{-1} for MeHg with HG. Also, a two-fold improvement of detection limits for Hg(II) and MeHg with GC-pyro-AFS has been reported using a home-modified AFS detector, with the inclusion of a quartz flow cell into the detector, which increases the concentration of Hg atoms in the detector.[141]

6.2. Speciation without chromatographic separation

Flow injection systems have been applied for Hg(II) and MeHg speciation. Online UV decomposition followed by CV-AFS has been applied to river water, preconcentrating both species in a column containing 2-mercaptobenzimidazole loaded on silica gel. Desorption of inorganic Hg is performed with 0.05 M potassium cyanide (KCN) and MeHg with 2 M HCl.[142] Also, acidic slurries of certified reference materials (fish), with traces of $K_2Cr_2O_7$ and a surfactant, are injected into the flow system. Mercury species are oxidised by adding a mixture of $KBr/KBrO_3$

Table 4 Selected references (in chronological order) for Hg speciation with AFS detection

Hg species	Sample matrix	Instrumental coupling	Analytical features	Reference (year)
EtHg, MeHg	Water	GC-AFS	Capillary column. Sample extraction: preconcentration onto sulfhydryl cotton fibre, KBr + $CuSO_4$ elution, back extraction with Cl_2CH_2	127 (1996)
MeHg	Fish certified reference material	GC-AFS	Capillary column. Sample extraction: $NaEt_4B$ derivatization + SPME[a]	128 (1998)
Hg(II), MeHg	Sediment CRM	HPLC-UV-CV-AFS	Reversed-phase column. Mobile phase: MeOH + 2 mercaptoethanol, pH 5, sample extraction: KOH/MeOH in ultrasonic bath, back extraction with Cl_2CH_2	123 (2001)
MeHg, EtHg	Food	GC-pyro-AFS	Capillary column. Sample extraction: alkaline extraction, enzymolysis, Cl_2CH_2/C_6H_8 extraction	138 (2002)
Hg(II), MeHg	Water	GC-pyro-AFS	Capillary column. Sample extraction: $NaEt_4B$ derivatization	132 (2002)
Hg(II), MeHg	Seafood	GC-pyro-AFS	Capillary column. Sample extraction: $NaEt_4B$ derivatization	133 (2004)
Complexes of MeHg, EtHg, PhHg	Fish	HPLC-CV-AFS	Reversed-phase column. Mobile phase: 7% MeOH and 0.05% 2-mercaptoethanol, pH 5, sample extraction: 6 M HCl + 1 M NaOH + MAE[b]	125 (2004)
MeHg, EtHg	Human biological samples	GC-pyro_AFS	Capillary column. Sample extraction: acid leaching, Cl_2H_2 extraction, $NaPR_4B$ derivatisation	135 (2007)

[a] Solid-phase microextraction. [b] Microwave assisted extraction.

heated at 50 °C for total Hg determination. In the absence of the oxidant, free Hg is measured.[143]

Another proposal to distinguish between Hg(II) and MeHg is photo-induced chemical/cold vapour generation, using only one reagent, formic acid. Under room natural light or UV irradiation, both species are converted to Hg^0, which are later detected by CV-AFS. In the absence of UV irradiation, only Hg(II) is measured, whereas MeHg is calculated by difference.[80,107]

Also, a new vapour generation system for Hg species (Hg(II) and MeHg) has been proposed, based on the UV irradiation of mercaptoethanol as an effective sample introduction unit for AFS detection. The new method has been validated with CRMs (BRC 463 tuna fish and BCR 580 estuarine sediment).[144]

There are some recent applications of CE directly coupled to AFS for speciation of Hg(I), MeHg, EtHg, and PhHg. The use of a hydrostatically modified electro-osmotic flow and a new developed interface allows online volatile species formation. The detection limits reported (6.8–16.5 μg L^{-1}) are in the same range as those obtained by chromatographic separation. So far, it has been successfully applied to Hg speciation in marine Certified Reference Materials (CRMs).[145] Another application of CE is the online hyphenation of flow injection, miniaturized CE and AFS. Hyphenation of FI-CE is achieved by a modified flow-through chamber as interface. The capillary outlet of CE was coupled to the AFS with a concentric "tube-in-tube" interface. A fast separation of Hg(II) and MeHg takes place in 60 s, with detection limits of 0.1 and 0.2 μg mL^{-1}, when applied to water samples.[146] The same authors have described an alternative of chip-based CE in connection to AFS detection, for speciation studies.[147]

7. Multielemental speciation

Multielemental speciation can be achieved either by coupling in tandem two AFS detectors, or using dual channel AFS instruments. The first approach has been employed for simultaneous determination of Se(IV) and Se(VI), As(III), As(V), DMA, and MMA in contaminated water samples.[86] Equally, two AFS detectors in series have been coupled for simultaneous determination of As(III), As(V), MMA and mercury species (Hg^{2+} and $MeHg^{+}$) in spiked natural fresh water samples.[148] On the other hand, dual channel instruments have been employed for the simultaneous speciation of As(III), As(V), Sb(III) and Sb(V) in traditional Chinese medicines, by control of acidity.[74]

8. Conclusions and future work

The use of AFS detection is widely extended for speciation of As, Se, Sb and Hg, for both routine and research studies, comprising more than a hundred scientific articles published in the last decade. Analytical features, such as low detection limits and wide linear calibration ranges, make AFS a suitable atomic detector in speciation studies, superior to AAS and equal to ICP-MS or ICP-AES, as long as single element speciation studies are considered. Compared to ICP techniques, AFS provides additional advantages: low acquisition and running costs, robustness and ease of operation.

Whilst future developments in the vapourisation step and the preference of the instrumentation for atomic fluorescence may emerge, it is the simplicity of the current instrumentation of both AFS and CV that is most appealing and ensures its continuing use for speciation studies.

There is no doubt that speciation studies will become more relevant, however, it is necessary to define adequate international standard methods so that data are transferable between different regimes. Other separation techniques may be added to the analysts' expertise, however, it is clear that AFS is an invaluable tool for the analyst.

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