Overview of speciation chemistry of arsenic

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Studies on the speciation of arsenic in environmental and biological samples are a subject of current interest. Because of the low level of arsenic species in real samples, many problems related with its speciation remain unresolved: species instability during sampling, storage and sample treatment, incomplete recovery of all species, matrix interferences, lack of appropriate certified reference materials and of sensitive analytical methods, etc. The continued development of new analytical procedures to solve some of these problems claim for an up-to-date knowledge of the recent publications. The analytical figures of merit, specially detection limits are given for most of the methods in order to afford comparison and judge possible applicability. These studies, which have been approached in many different ways, would lead to knowledge that is determinant in the understanding of the cycle of this element in the environment and of its physiological and toxicological behaviour in the living organisms.

ARSENIC occurs naturally in a wide range of minerals, which, together with a once widespread use of arsenic in pigments, insecticides and herbicides, represent the major sources of arsenic in natural waters.

About 70% of all arsenic used is in pesticides, principally the following.

(1) Monosodium methane arsenate (MSMA) – $HAsO_3CH_3Na$;
(2) Disodium methane arsenate (DSMA) – $Na_2AsO_3CH_3$;
(3) Dimethylarsinic acid (cacodylic acid) – $(CH_3)_2\ AsO_2H$;
(4) Arsenic acid – H_3AsO_4.

The other uses of arsenic and arsenic compounds are in wood preservatives, glass manufacture, alloys, electronics, catalysts, feed additives and veterinary chemicals[1].

Speciation analysis of an element in a water sample may be defined as the determination of the concentration of the different physico-chemical forms of the element which together make up its total concentration in the sample. The individual physico-chemical forms may include particulate matter and dissolved forms such as simple inorganic species, organic complexes and the element adsorbed on a variety of colloidal particles[2]. The speciation of arsenic in environmental materials is of interest because of the differing levels of toxicity exhibited by the various species. The major arsenic species found in environmental and clinical samples are arsenite As(III), arsenate As(V), arsenious acids (H_3AsO_3, $H_2AsO_3^-$, $HAsO_3^{2-}$), arsenic acids (H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$), dimethylarsinate (DMA), monomethylarsonate (MMA), arsenobetaine (AB) and arsenocholine (AC).

These forms illustrate the various oxidation states that arsenic commonly exhibits (– III, 0, III, V) and the resulting complexity of its chemistry in the environment.

Among the arsenic compounds in the environment, of particular interest is arsenite, which is 10 times more toxic than arsenate and 70 times more toxic than the methylated species, DMA and MMA[3]. DMA and MMA are moderately toxic, whereas AB and AC are virtually non-toxic[4]. These facts indicate why it would be of priority interest to develop methods for the selective determination of As(III).

Speciation reactions in water

In aqueous systems, arsenic exhibits anionic behaviour. In aerobic waters, arsenic acid predominates only at extremely low pH (< 2); within a pH range of 2 to 11, it is replaced by $H_2AsO_4^-$ and $HAsO_4^{2-}$. Arsenious acid appears at low pH and under mildly reduced conditions, but it is replaced by $H_2AsO_3^-$ as the pH increases. Only when the pH exceeds 12 does $HAsO_3^{2-}$ appear. At low pH in the presence of sulphide, $HAsS_2$ can form; arsine, arsine derivatives and arsenic metal can occur under extreme reducing conditions[5]. Figure 1 shows the speciation of arsenic under varying pH and redox conditions[6].

Since it forms anions in solution, arsenic does not form complexes with simple anions like Cl^- and SO_4^{2-} as do cationic metals. Rather, anionic arsenic complexes behave like ligands in water. Arsenic forms bond with organic sulphur, nitrogen and carbon. As(III) reacts with sulphur and sulphydryl groups such as cystine, organic dithiols, proteins and enzymes, but it does not react with amine groups or organics with reduced nitrogen constituents. On the other hand, As(V) reacts with reduced nitrogen groups such as amines, but not sulphydryl groups. Carbon forms organoarsenicals with both the trivalent and pentavalent forms[1]. The complexation of arsenic (III and V) by dissolved organic matter in natural environments prevents sorption and coprecipitation with solid-phase organics and in-organics; essentially, it increases the mobility of arsenic in aquatic systems and in the soil[7]. Figure 2 shows a typical cycle of arsenic in a stratified lake[8].

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Biotransformation

Many aquatic organisms are capable of accumulating arsenic and may catalyse the oxidation of arsenite to arsenate while also promoting the formation of methylarsines through biomethylation reaction[9]. Certain diatoms are known to reduce arsenate to arsenite and dimethylarsinic acid[10]. The production of methylarsines by methogenic bacteria under aerobic conditions has been demonstrated by McBride and Wolfe[11]. This could conceivably occur in sediments, with the species produced being subsequently released to the overlying water. McBride and Wolfe[11] showed that dimethylarsinic acid and methylarsonic acid ($CH_3AsO(OH)_2$) are intermediates in the reductive methylation of inorganic arsenic to dimethylarsine. Ridley *et al.*[9] however, proposed reaction of molecular oxygen with volatile arsines as the means of formation of these acids. Organic arsenic compounds are also known to be present in marine animals, and recently the composition and structure of one such compound, arsenobetaine, isolated from the tail muscle of a western rock lobster was described[12]. It is possible that such compounds could also find their way into natural waters.

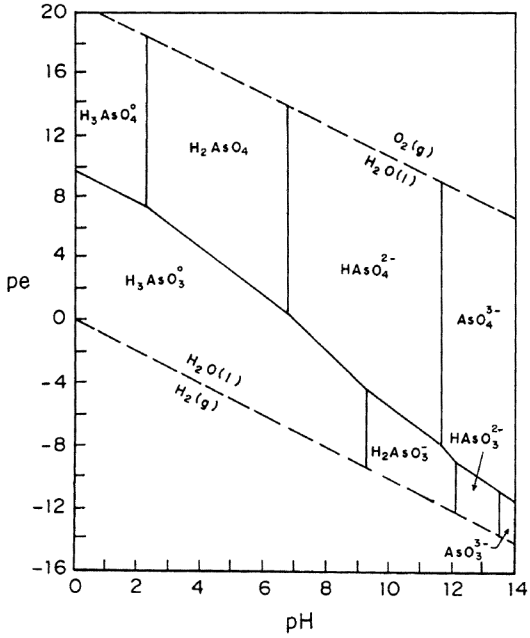

Figure 1. Pe–pH diagram of arsenic in water at 25°C.

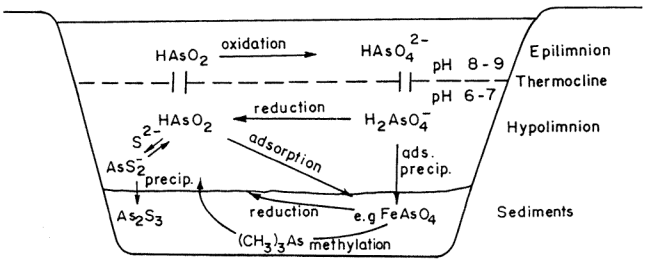

Figure 2. Local cycle of arsenic in a stratified lake.

Arsenic compounds are methylated by bacteria and fungi to yield dimethyl and trimethylarsines by a mechanism that involves the replacement of substituent oxygen atoms by methyl groups[13]. Methylation is thought to be a detoxification mechanism for the micro-organisms[14] and is important in the transfer of arsenic from the sediment to the water and atmosphere.

McBride and Wolfe[11] reported that arsenate could be reductively methylated to dimethylarsine by *Methanobacterium* under anaerobic conditions. The reaction (Figure 3) given by Saxena and Howard[15], involves the initial reaction of arsenate to arsenite. Methylarsonic acid, formed by the methylation of arsenite, is reductively methylated to dimethylarsinic acid, which is reduced to dimethylarsine. Methylcobalamin is the methyl-donor in the reaction.

Under acidic conditions, the sewage fungi *Candida humicola* transform arsenate to trimethylarsine. Lesser amounts of trimethylarsine are also formed by the incubation of the fungi with arsenite, methylarsonate and dimethylarsinate. These may be intermediates in the reduction and alkylation of arsenate to trimethylarsine[16]. Fungi capable of generating trimethylarsine from the pesticides monomethylarsonate and dimethylarseine include *Candida humicola*, *Gliocaninum roseum* and a strain of *Penicillium*[17]. The rate of formation of trimethylarsine by *C. humicola* and other molds decreases as the mold reaches its resting stage[18].

The toxic alkylarsines are volatile, with a distinct garlic-like odour and are rapidly oxidized to less toxic products in the atmosphere. One such product dimethylarsinic acid has been shown to be an intermediate in the

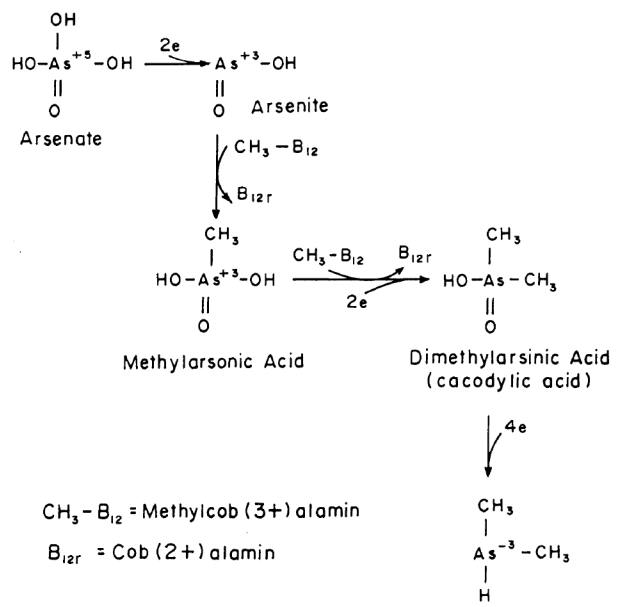

Figure 3. Reductive methylation of arsenite to dimethylarsine.

synthesis of dimethylarsine from arsenic salts[19]. Figure 4 shows the biological cycle for arsenic[19].

The methylation of arsenic is important because of the extremely toxic products that result. Also, this process transfers arsenic from sediments back to the water column in aquatic systems, increasing arsenic mobility in the environment. Biotransformation of arsenic can produce highly volatile compounds such as arsine (AsH_3), dimethylarsine ($HAs(CH_3)_2$) and trimethylarsine ($As(CH_3)_3$).

Methods of determination of speciation of arsenic

Voltammetric method

In the determination of arsenic in water samples, no extraordinary precautions are needed in taking samples or preventing contamination. The only concern is the preservation for speciation because As(III) can rapidly oxidize to As(V), but this should not happen with immediate analysis[20].

Feldmann[21] reported successful preservation of As(III) in standards with ascorbic acid. Sadana[22] failed to reproduce his work. As a test of the effectiveness of hydrazinium chloride as preservative for As(III), the refrigerated samples were analysed at regular intervals over a period of 20 days. No significant loss of As(III) was observed. The unpreserved sample showed approximately 50% loss of As(III) within the first 2 days of sample collection and a total loss over a period of approximately 6 days.

Arsenic has been determined by several electrochemical techniques. The differential pulse polarographic technique (DPP)[23–25] had been used extensively. The polarographic methods are not sensitive for the determination of arsenic at ultratrace concentration. Stripping voltammetric methods are better suited because of their *in situ* pre-concentrating capability. The anodic stripping voltammetry (ASV) methods using platinum and gold electrodes[26–28] and cathodic stripping voltammetry (CSV) method using a glassy-carbon electrode[29] have been used.

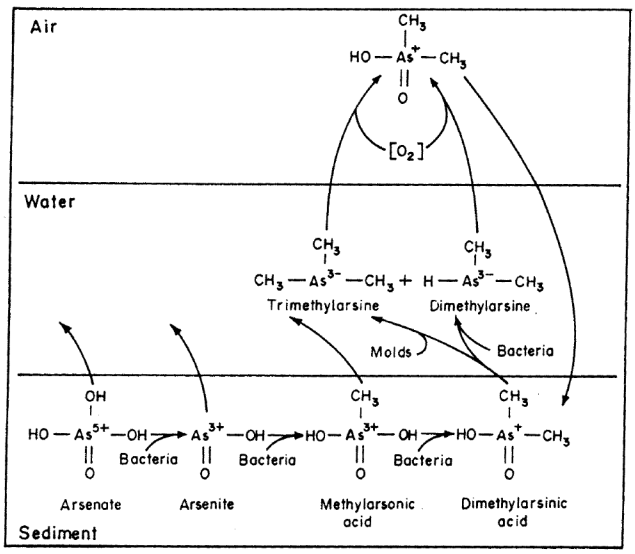

Figure 4. Biological cycle of arsenic.

Sadana[22] determined arsenic in drinking water with Cu(II) by differential pulse cathodic stripping voltammetry (DPCSV) using hanging mercury drop electrode (HMDE) as working electrode and Ag/AgCl as reference electrode. The optimized analytical conditions are 0.75 M hydrochloric acid, 5 ± 1 μg/ml Cu^{2+} concentration and – 0.6 V deposition potential. The detection limit of this method is 1 ng/ml. The total arsenic in water is determined by reducing As(V) to As(III) by heating the sample with concentrated hydrochloric acid and 48% hydrobromic acid in a steam bath set at 95–100°C for 45 min. The solution is cooled to room temperature and diluted with 0.25% (w/v) hydrazinium chloride solution and CSV is carried out.

In the determination of total arsenic in water sample, Forsberg *et al.*[26] reduced As(V) to As(III) which is necessary because As(V) is electro-inactive, involved heating As(V) with sodium sulphite (Na_2SO_3) in concentrated acid solutions. The optimized analytical conditions are 1 M hydrochloric acid or 1 M perchloric acid, and 0.5 V as the suitable deposition potential. Gold or platinum and Ag/AgCl used as working and reference electrodes, respectively. The detection limit of both DPASV and ASV is 0.02 ng/ml. Holak[30] developed a CSV procedure for the determination of arsenic in acidic solutions containing Se(V) by using HMDE. The latter does not suffer from the disadvantage of solid electrodes and its use in routine stripping voltammetry is in practice. Copper interferes severely in this method.

Henze *et al.*[31] studied the speciation of As(V) and As(III) by CSV in fresh water samples. Ten ml water sample is mixed with 100 μl hydrogen peroxide and 50 μl concentrated sulphuric acid and digested in a UV digestor to remove organics. The sample is then analysed by CSV at a hanging mercury drop electrode and a double-junction Ag/AgCl/3 M KCl reference electrode and a glassy-carbon auxiliary electrode. After deaeration, preconcentration is carried out at – 550 mV and stripping is carried out at scan rate of 25 mV/s with a pulse amplitude of – 50 mV. The supporting electrolyte contained 0.4 M H_2SO_4, 0.22°M D-mannitol, 10 mg/l Cu(II) and 70 mg/l Se(IV). The detection and determination limits are 0.52 and 0.93 μg/l As, respectively. As(III) is similarly determined, except that the UV-irradiation of the sample and the use of mannitol in the supporting electrolyte are omitted.

Pretty *et al.*[32] presented an on-line, ASV flow cell with detection by inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) for As(III) determination. With this method, polyatomic interferences which arise

from chloride in sample matrices are eliminated. The detection ICP-AES has insufficient sensitivity in many cases, lower detection limits can be obtained with a hydride generator coupled by ICP. The detection by ICP-MS enhances the signals.

Spectrophotometric method

Howard and Arbab-Zavar[33] used silver diethyldithiocarbamate (SDDC) spectrophotometric procedure for the determination of arsenite and arsenate. This procedure has interferences from trace metals and methylated arsenic species, but these can be prevented using chelation, solvent extraction or ion-exchange. Palanivelu *et al.*[34] described a highly sensitive spectrophotometric method for the determination of arsenite and arsenate. Absorbance is measured with a Carl Zeiss PMQII spectrophotometer with 10 mm quartz cells. The determination is made as arsenic tri-iodide after an extractive separation into benzene. As(V) is determined by measuring total arsenic after the reduction of arsenate with potassium iodide. The results are more sensitive than those based on reaction with SDDC, and superior to the fluorescence method based on the use of rhodamine-B. This method has the advantage of lack of interferences and permits precise determination of trace amounts of arsenic in natural and synthetic samples.

Tamari *et al.*[35] presented a new co-precipitation method for the spectrophotometric determination of arsenite and arsenate in groundwater. Both species are co-precipitated with thorium(V) hydroxide at pH 9; after centrifugation and dilution with hydrochloric acid the absorbance is measured on a spectrophotometer by the usual SDDS method.

Chatterjee *et al.*[36] analysed As species, arsenite and arsenate, spectrophotometrically using AgDDTC/$CHCl_3$/hexamethylenetetramine as absorbing solution.

Electrophoresis

Schlegel *et al.*[37] presented a modification of the high performance capillary electrophoresis (CE) and capillary zone eletrophoresis (CZE), which used two different detection systems, photometric and conductimetric. The CZE shows excellent suitability for arsenic speciation and the separation of different species is possible. With the photometric detector As(III), As(V) and DMA are determined and with the conductometric detector As(V), DMA, *p*-aminobenzene arsonate (ABA) and phenylarsonate (PhAs).

Another CE technique for determining As(III), As(V), MMA and DMA is presented by Lin *et al.*[38]. They introduced a different detection system (ICP-MS) and developed a novel interface which is based on a direct injector nebulizer (DIN). This interface can be used under different CE conditions and it allows for the DIN to be optimized independently from the CE system to give the most efficient sample introduction into the ICP-MS. This method therefore combines the high separation efficiency of the CE with high elemental sensitivity of the ICP-MS.

Magnuson *et al.*[39] proposed a method to determine arsenic species using CE with hydrodynamically modified electro-osmotic flow. The detection is by hydride generation inductively coupled plasma mass spectrometry (HG-ICPMS). The method is used to determine As(III), As(V), monomethylarsonic acid and dimethylarsinic acid.

Vanifatova *et al.*[40] determined arsenite by CZE with the direct photometric detection. As(III), As(V) and DMA are separated in a fused-silica capillary at 25 kV, with 20 mM phosphate buffer of pH 7–10.8 as electrolyte and detection at 200 nm. Samples are injected hydrodynamically at the anodic end at 3.45 kpa. A 50 s injection time and a pH of 10.6 are optimal. Calibration graphs are linear for 0.35–2.5 mg/l As(III), 1–5 mg/l As(V) and 1.3–5 mg/l DMA and the detection limits are 0.15, 1.1 and 1.3 mg/l, respectively.

Tian *et al.*[41] used movable reduction bed generation system as an interface for CZE and ICP-AES for arsenic speciation analysis. The sample (400 nl) is injected hydrostatically into a fused-silica capillary. Separation of the As species is effected at 15 kV at 50 mM phosphate buffer of pH 6 (buffer A) as the electrolyte. The elute from the CZE column is mixed with a stream (10 μl/min) of buffer A before impinging upon the surface of the tape coated with potassium borohydride/tartaric acid (1 : 3) housed in a glass chamber. The hydride generated is swept from the chamber by an Ar carrier gas flow and analysed for As by ICP-AES at 193.7 nm. The detection limits are 0.32 μg/ml for As(III), As(V) and DSMA and 0.35 μg/ml for dimethylarsinic acid.

Speciation by hydride derivatization

Figure 5 shows the schematic representation of hydride derivatization experiment[42].

Hydride derivatization volatilizes the compounds, allowing their trapping on an analytical column, elution and separation, atomization and detection. Atomization is necessary for the common detection methods like atomic absorption spectrometry (AAS) and atomic fluorescence spectrometry (AFS). The water trap before the packed column protects it from water and prevents water interference in AFS.

$NaBH_4$ volatilizes inorganic As(III) and As(V) by formation of arsine and $Me_nAsO(OH)_{3-n}$ ($n = 1$ to 3) by formation of methylarsenic(III) hydrides and trimethylarsenic(III) (Table 1)[43].

The general (eq. (1)) and sample (eq. (2)) reactions for derivatization of methylarsenic compounds are not balanced because the fate of $NaBH_4$ is unknown.

$$Me_nAsO(OH)_{3-n} + NaBH_4 \rightarrow Me_nAsH_{3-n}, \quad (n = 1 \text{ to } 3) \tag{1}$$

$$Me_3AsO + NaBH_4 \rightarrow Me_3As. \tag{2}$$

Me_4As^+, arsenosugars (AS) and arsenoamino acids are not directly derivatized by $NaBH_4$ and must be separated and decomposed into inorganic arsenic before hydride derivatization[44]. Cullen and Reimer[45] have reviewed many aspects of inorganic arsenic and methylarsenic compounds in the environment.

A problem with the methods summarized in Table 1 is that different arsenic compounds have different optimum pH values for derivatization and that at constant pH (e.g. pH 0.5) they have different sensitivities. This is a common problem in simultaneously derivatizing several compounds. Le *et al.*[46] solved this problem and obtained excellent reproducibility and sensitivity for arsenic compounds by adding 2% aqueous L-cysteine ($HSCH_2CHNH_2CO_2H$) along with sodium borohydride. The reasons for the improvements are that L-cysteine (RSH) reduces As(V) compounds to As(III) analogues and that its anion (RS^-) binds the resulting arsenic (III) compounds before hydride derivatization (eqs (3–5)). However, this procedure does not distinguish inorganic As(III) and As(V) (eq. (3)).

$$AsO(OH)_3, As(OH)_3 \rightarrow As(SR)_3 \rightarrow AsH_3, \tag{3}$$

$$MeAsO(OH)_2 \rightarrow MeAs(SR)_2 \rightarrow MeAsH_2, \tag{4}$$

$$MeAsO(OH) \rightarrow Me_2As(SR) \rightarrow Me_2AsH. \tag{5}$$

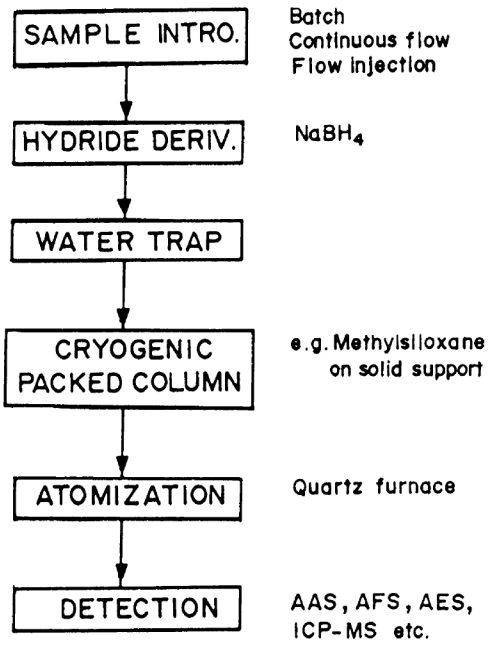

Figure 5. Schematic representation of hydride derivatization experiment, including trapping, atomization and detection. AAS, atomic absorption spectrometry; AFS, atomic fluorescence spectrometry; AES, atomic emission spectrometry; and ICP-MS, inductively coupled plasma-mass spectrometry.

Speciation using microwave digestion

Arsenic in food sample is determined[47] using microwave pressure decomposition. A mixture of sample in 1.8 g fruit or vegetable, 3 ml of 30% hydrogen peroxide, 0.5 ml 65% nitric acid and 1.5 ml H_2O is subjected to a ten-step microwave digestion at 0–650 W and diluted to 10 ml, containing 5 ml digestate, 1 M hydrochloric acid and 10 g/l KI/ascorbic acid. This is then subjected to hydride-generating AAS in an air/acetylene flame, and later to hydride-generating AAS in an air/acetylene flame at 193.2 nm with 1 ml/min 6 g/l sodium borohydride solution and 5 M hydrochloric acid. Organic arsenic is determined similarly after reduction of 4 ml digestate with 2 ml 2.2 M $Na_2S_2O_8$ and 1 ml 40 g/l sodium fluoride for 10 min at 800 W. Recoveries of As as As(III), dimethylarsine and AB from pork are 83–106%.

The low-power microwave-assisted extraction of As species from muscles is optimized by applying a sequential Doehlert design[48]. The effects of microwave power exposure time and composition of the methanol/H_2O extraction medium are investigated. The extractions are performed using a focused microwave digester, 500 mg lyophilized mussel tissue and 20 ml extraction solvent. The maximum yield of 85% for AB, AS, MMA, DMA and As(V) is achieved using a power of 40 W, an exposure time of 4 min and methanol/H_2O at 11.9. The extracts are cleaned up by SPE using a C_{18} catridge and analysed by LC-UV-hydride generation ICP-MS. The proportions of the arsenic species with respect to the total arsenic are 68% AB, 14% AS plus MMA and 2% DMA.

Chromatographic methods of speciation

High performance liquid chromatography

The method most commonly employed for the separation of arsenic species is high performance liquid chromatography (HPLC) in its various forms. The molecular forms of As which are subjected to speciation analysis are the anions, As(III), As(V), MMA and DMA or the cations AB, AC or trimethylarsenic (TMA). The separation techniques used are anion-exchange HPLC with either isocratic or gradient-step elution or cation-exchange HPLC

Table 1. Overview of speciation of arsenic compounds by hydride derivatization

Analyte	pH	Product
$As(OH)_3$	– 1 to 7	AsH_3
$AsO(OH)_3$	> 0.3	AsH_3
$MeAsO(OH)_2$	0.3–1 (HCl)	$MeAsH_2$
$Me_2AsO(OH)$	0.3–1 (HCl)	Me_2AsH
Me_3AsO	0.3–1 (HCl)	Me_3As

with isocratic elution. Ion-pair HPLC has also been used. In order to separate arsenic anions and cations in a single run, a column-switching system involving a combination of anion-exchange and reversed-phase separation has been developed. After separation by HPLC, the As species are detected on-line by several detection systems such as ultraviolet (UV), AAS, hydride generation-atomic absorption spectrometry (HG-AAS), ICP-AES, ICP-MS or off-line by electrothermal atomic absorption spectrometry (ETA-AAS). Hansen *et al.*[49] applied HPLC-AAS coupled on-line system for the speciation of arsenic species. As(III), As(V), MMA and DMA are separated from each other and from the co-injected cationic arsenic compounds on an organic polymeric anion-exchange column with 0.1 M carbonate at pH 10.3 as the mobile phase. AB, AC and TMA are separated from each other and from the co-injected anionic species on a silica-based cation-exchange column, with pyridine at pH 2.65 as the mobile phase.

For signal enhancement a slotted tube atom trap (STAT) is installed. The atom trap is made from a quartz tube and is attached by screws to the burner head. The interfacing is established by a vented polytetrafluorethylene (PTFE) capillary tube connecting the HPLC column to the nebulizer of the atomic absorption spectrometer and using a H_2–Ar flame. The detection limits are sufficiently low for the selected applications, although these detection limits are 150–470 times worse than those from the ICP-MS.

The methods that employ hydride generation between the HPLC and AAS present better sensitivity. Their disadvantage is that only molecules that produce volatile arsines can be detected. Hakala and Pyy[50] studied arsenic exposure and its monitoring in urine samples by using a system with on-line HPLC-HG-AAS. The separation of As(III), As(V), MMA and DMA is carried out on a C_{18} reversed-phase column with 10 mM tetrabutylammonium (TBA), 20 mM phosphate at pH 6.0 as the mobile phase. Because AB and AC do not generate hydrides, a pre-oxidation to an inorganic arsenical is necessary, as by the on-line combination of $K_2S_2O_8$ with UV-light.

Lopez *et al.*[51] have incorporated the on-line thermo-oxidation to the speciation of As(III), As(V), MMA, DMA, AB and AC. They used an anion column with 17 mM phosphate at pH 6.0 as the mobile phase. The effluent of the HPLC was merged with a persulphate stream before entering the thermo-reactor consisting of a loop of PTFE tubing dipped in a powdered-graphite oven heated to 140°C. After cooling in ice-bath, hydrochloric acid and sodium borohydride are added on-line to generate the arsine. A similar HPLC system, with the same chromatographic column and with a post-column reaction to achieve complete formation of volatile arsines from the methylated species and As(V), is developed for the speciation of As(III), As(V), MMA and DMA[52].

In HPLC-HG-ICP-AES only a few researchers use hydride generation to detect arsines in the gas phase. A gas–liquid separator was used by Rauret *et al.*[53] to minimize the volume of solution reaching the plasma torch and to improve the separation of volatile hydrides. They used a silica-based anion-exchange column to separate the arsenic species As(III), As(V), MMA and DMA with phosphate buffer, pH 6.75 as the mobile phase. Rubio *et al.*[44] developed the separation of As(III), As(V), MMA, DMA, AB and AC on an anion-exchange column using a phosphate buffer as the mobile phase. On-line UV-photo-oxidation is used by these authors at the exit of the chromatographic column for the decomposition of AB and AC to As(V).

Beauchemin *et al.*[54] coupled various forms of HPLC with ICP-MS for arsenic speciation. They studied ion-pairing and ion-exchange HPLC to separate As(III), As(V), MMA, DMA and AB. They found that anion exchange has resolution inferior to ion-pairing chromatography, but it appears to be less susceptible to matrix interferences.

Demesmay *et al.*[55] used an ICP-MS detector coupled to an HPLC system to determine As(III), As(V), MMA, DMA, AB and AC. The interface is established through PTFE capillary tubing. They used an anion-exchange column with a mobile phase of phosphate buffer with 2% acetonitrile. To reach an optimum separation of the six arsenic species, an ionic-strength step gradient is necessary.

Gas chromatographic methods of speciation

Beckermann[56] presented an elegant method for the determination of MMA and DMA in biological samples. Most of the methods proposed for this speciation are based on conversion by sodium borohydride of arsenic into the corresponding methylarsine compounds, but their volatilities make it necessary to convert MMA and DMA into stable derivatives. With this method, the methylarsenic acids are derivatized with thioglycolic acid methylester, to yield lipophilic species which can be determined by gas chromatography (GC) using a flame ionization detector (FID).

Another technique presented by two different groups of authors[57,58] is based on selective hydride generation, liquid-nitrogen-cooled trapping and gas chromatography with helium-discharge type photoionization detection (PID). It is applied to the simultaneous determination of As(III) and Sb(III) under weak-acid conditions and to As(III), As(V), Sb(III) and Sb(V) under strong acid conditions.

Talmi *et al.*[59] presented a method consisting of gas chromatography coupled with a microwave emission spectrometric (MES) system detector for arsenic and antimony determination in environmental samples. The

analytical procedure is based on the following steps: sample pretreatment, co-crystallization of As(III) and Sb(III) with thionalide and reaction of the dry precipitate with phenyl magnesium bromide (PMB). When the phenylation has been completed, the excess of PMB is decomposed to prevent oxidation of Ph_3As, then a portion of the organic layer is injected into the column with a gradient programme and determined by MES.

Ion-exchange chromatography

Ricci *et al.*[60] presented a technique that separates the species using ion-exchange chromatography followed by continuous hydride generation and atomic absorption detection. The separation of the arsenic species As(III), As(V), MMA, DMA and *p*-aminophenylarsonate (*p*-APA), is achieved by using a standard Dionex 3 × 500 mm anion-separator column. A mixture of $NaHCO_3$ and $Na_2B_4O_7$ is used as the mobile phase for the separation of As(V), MMA and *p*-APA, and $Na_2B_4O_7$ is used in the case of As(III) and DMA. The effluent exiting the column led via microbrode tubing directly into a hydride generator-AAS detection system.

McGeehan and Naylor[61] described a direct simultaneous measurement of arsenic and selenium inorganic species using a suppressed-ion chromatography system with a Dionex 4000*i*. A sodium carbonate–sodium bicarbonate solution is used as the mobile phase and an electrochemical and a conductimetric detector are used in series with a dual-channel integrator to quantify As(III) and As(V). Although suppressed ion chromatography (SIC) achieves lower detection limits than IC, neither has sufficient sensitivity for the analysis of many environmental samples. The major advantage of SIC is its ability to separate and quantify the ionic species of arsenic and selenium without oxidation/reduction pretreatments.

Pantsar-Kallio *et al.*[62] proposed a method to determine arsenic species present in the water sample using ion-exchange-ICP-MS. A good separation of As(III) and As(V) is obtained and the method can also be used to determine the less toxic DMA and MMA, as well as the sum of AB and AC which do not normally occur in drinking water. For all the species the detection limits are 0.4–0.5 μg/l.

Ion-exclusion chromatography

Hemmings and Jones[63] presented an ion-exclusion chromatography (IEC) method to measure As(III) and As(V). Any iron is an important interference, which is removed by a cation-exchange procedure before the separation of the arsenic species by using a Dionex 2010*i* ion chromatograph. The arsenic speciation is carried out by using an HPICE-AS1 ion-exclusion column with a photometric detector. The main problem is the lack of sensitivity of this detector for the determination of arsenic species at trace levels.

Supercritical fluid chromatography

Laintz *et al.*[64] described a novel technique based on a simultaneous separation and quantification of As(III) and As(V) (after reduction with potassium iodide and sodium-thiosulphate), achieved by extraction with lithium bis(trifluoroethyl)dithiocarbamate followed by supercritical fluid chromatography (SFC) with FID. The extraction technique reduces organic matrix interferences and preconcentrates the metal species by conversion into chelates suitable for SFC analysis.

As speciation in refractory

Battencourt *et al.*[65] presented the speciation of refractory arsenic which does not form hydrides. The speciation scheme is proposed based on solvent-extraction, HPLC and dynamic-flow fast atom-bombardment MS. Solvent extraction with methanol was followed by ion-exchange weak cationic (Lewatit CNP 80; Na form) and strong cationic (Amberlite IR-120; H^+ form). The speed of the concentration step is increased through freeze-drying and rotary evaporation. The low photo-oxidation step is suppressed and the detection of total As is performed using gas-furnace AAS or hydride generation of total digests. For the final isolation step HPLC on Rp-18 columns are used. The final identification was performed by LC fast atom-bombardment MS.

As speciation using resin

Suzuki *et al.*[66] removed As(III) and As(V) by a porous spherical resin loaded with monoclinic hydrous zirconium oxide. Amberlite XAD-7 is saturated with methanolic zirconium oxy chloride ($ZrOCl_2$) solution, dried and then stirred for 5 h with 28% NH_3. After dilution with H_2O and separating the precipitate, the pH of the resin water pulp is made 2 for hydrothermal treatment at 150°C for 15 h. The resin has good sorption characteristics, with a logarithmic distribution ratio of 2–4, depending on the pH of the solution and the oxidation state of the As.

The various instrumental techniques[67–71], the species determined and the detection limits are listed in the Table 2.

As speciation using micro-organisms

Many micro-organisms such as algae, fungi, yeast and bacteria have been used for biosorption of toxic elements

Table 2. Instrumental techniques for arsenic speciation

Method	Arsenic species determined	LOD ($\mu g l^{-1}$)	Reference
Spectrophotometric	As(III), As(V)	100, 100	33
	As(III), As(V)	0.03, 1	34
	As(III), As(V)	–	35
FIA-HG-AAS	As(III), As(V), MMA, DMA	–	67
FIA-HG-ICP-AES	As(III), As(V), MMA, DMA	–	
HPLC-AAS	As(III), As(V), MMA, DMA, AB, AC, TMA	1100, 1400, 1400, 700, 300, 500, 400	49
HPLC-HG-AAS	As(III), As(V), MMA, DMA	1, 1.6, 1.2, 4.7	50
HPLC-UV-HG-AAS	As(III), As(V), MMA, DMA, AB, AC	5, 8, 6, 6, 4, 4	51
	As(III), As(V), MMA, DMA	0.5, 0.5, 0.5, 0.5	52
IC-HG-AAS	As(III), As(V), MMA, DMA, P-APA	4, 20, 3.2, 6.5, 9.3	60
SIC-electrochemical	As(III)	114	61
SIC-conductimetric	As(V)	120	
IEC-PID	As(III), As(V)	> 3000	63
HPLC-HG-ICP-AES	As(III), As(V), MMA, DMA	3.5, 9.2, 3.8, 21.3	53
Ion-exchange ICP-MS	As(III), As(V)	0.4, 0.4	62
HPLC-UV-HG-ICP-AES	As(III), As(V), MMA, DMA, AB, AC	2.6, 9.6, 13, 9.8, 7.9, 6.1	44
HPLC-ETAAS	As(III), As(V), MMA, DMA, TMA	–	68
HPLC-HG-MIP-AES	As(III), As(V), MMA, DMA	1, 5, 1.2, 6	69
HPLC-ICP-MS	As(III), AB	1, 5	54
	As(III), As(V), MMA, DMA,	4.9, 6, 3.6, 1.2	70
	As(III), As(V), MMA, DMA, AB, AC	0.5, 0.3, 1, 1, 0.5, 0.5	55
HPLC-USN-AFS	As(III), As(V), MMA, DMA	0.14, 0.2, 0.08, 0.08	71
CZE-UV	As(III), As(V), DMA	90, 60, 120	37
CZE-ICP-MES	As(III), As(V), DSMA, Cacodylic acid	320, 320, 320, 350	41
CZE	As(III), As(V), DMA	150, 1100, 1130	40
CE-ICP-MS	As(III), As(V)	0.1, 0.02	38
GC-FID	As(III), As(V)	10, 10	56
HG-GC-PID	As(III), As(V)	0.0018	57
		0.0008	58
GC-MES	As(III)	0.05	59
SFC-FID	As(III), As(V)	87.5, 87.5	64
Voltammetric ICP-AES	As(III)	5	32
Voltammetric ICP-MS	As(III)	0.13	
DPASV/ASV	As(III), As(V)	0.02, 0.02	26
DPCSV	As(III), As(V)	1, 1	22
CSV	As(III), As(V)	0.52, 0.52	31

by binding to the cell surface and intra-cellularly. This property has been applied largely to the pre-concentration of metal ions, mainly in industry, the environment, etc.[72], but its application to metal speciation has recently been proposed. The capacity of some algae to accumulate arsenic has been known for several years[73] and has been used to eliminate this element from industrial effluents[72]. Yamaoka *et al.*[74–76] studied the accumulation of arsenic by *Duniella* sp. and the effect of various elements on this accumulation. Figure 6 (ref. 77) shows the analytical procedure for the As(III) retention by *Chlorella vulgaris*. The capacity of the alga *C. vulgaris* to transform inorganic arsenic compounds[78–80] and to oxidize As(III) to As(V)[81,82] has been evaluated. Goessler *et al.*[83] studied the uptake of arsenic compounds by three *Chlorella* species. Bioaccumulation and biomethylation of inorganic arsenic by the marine alga *Polyphysa peniculus*[84] and the blue-green alga *Nostoc* sp.[85] are also investigated. Five bacteria (*Proteus* sp., *Escherichia coli*, *Flavobacteriam* sp., *Corynebacterium* sp. and *Pseudomonas* sp.), are used to determine arsenic uptake and distribution into the cells[86]. According to the work mentioned above, the relationship between different micro-organisms and arsenic is widely demonstrated, but this fact has not yet been exploited as a practical way for arsenic speciation.

Conclusions and future prospects

From this survey it can be concluded that the analytical techniques available for the detection and speciation of arsenic are diverse. Each approach possesses both advantages and disadvantages that must be considered with respect to the scope of the study and also the laboratory facilities available. Adequate quantitative determination of complex matrices requires the establishment of efficient separation and pre-concentration processes, good

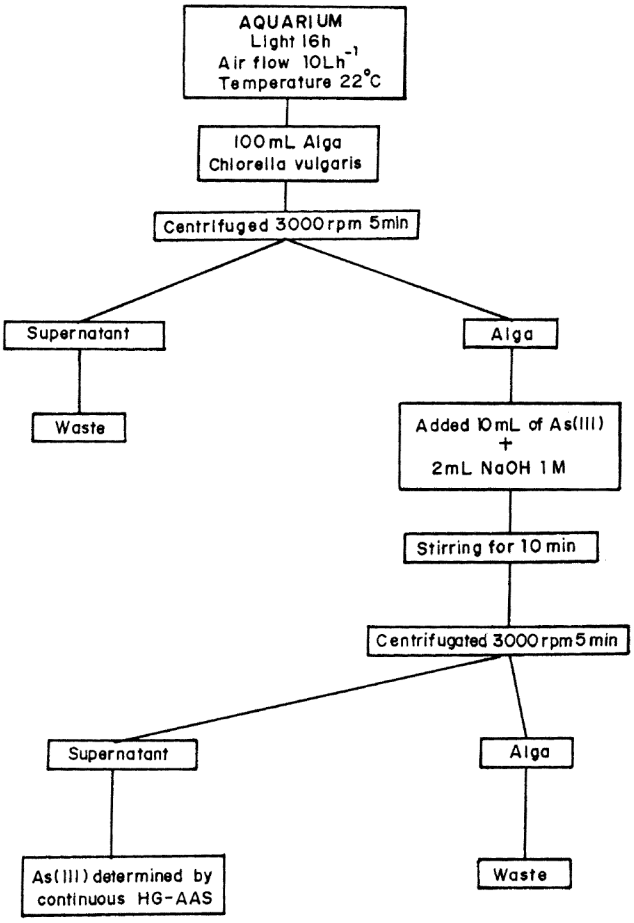

Figure 6. Analytical procedure for the As(III) retention by *Chlorella vulgaris*.

recovery in clean-up procedures and precision and accuracy controls. Much work has to be conducted in order to understand first the natural distribution cycle of the element (the dynamics of its mobility through ecosystems) and to compare it with documented perturbations stemming from man's technological inputs and their impact on the public health. A balanced interdisciplinary approach is necessary, formed by analytical chemists, specialists in life sciences, statisticians, ecologists, etc. These multifaceted efforts would be able to respond to questions regarding the biotransformation and redox reactions occurring in biological media as well as the interactions that could take place in the environment between arsenic compounds and other natural products to produce new organoarsenicals of perhaps totally different toxicity.

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