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DETERMINATION OF ARSENIC SPECIES IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

(Technical Report)

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Determination of arsenic species in biological and environmental samples (Technical Report)

Abstract - Identification and quantitative determination methods of arsenic species in environmental and biological samples are discussed. Isolation followed by molecular spectroscopic determination is necessary for rigorous identification while element-specific detections coupled with separation techniques are the choice for quantitative determination. Analytical figures of merit are given for the methods applicable to a specific matrix evaluating hydride generation technique, high performance liquid chromatography coupled with atomic absorption, ICP atomic emission, ICP mass spectrometric detection, thin layer and gas chromatography with atomic absorption spectrometric detection and other methods. Sample preparation is the area that requires attention as it still represents the main source of problems including matrix interference, incomplete recovery and analyte instability.

1. INTRODUCTION

Arsenic is widely distributed in the biosphere. It occurs in sea water at a level of about 2μg/kg [1,2], but concentrations vary in fresh water with values of a few μg/kg being reported[3].

In ground water, it sometimes occurs at concentrations exceeding 21mg/kg [3-8]. Arsenic occurs in the earth's crust at an average of 2mg/kg and in normal soils at levels ranging from 1-40mg/kg [3]. It also occurs in the atmosphere through burning of fossil fuels and smelting of non-ferrous ores, as well as naturally through volcanism and from the oceans by bubble bursting. Terrestrial plants and freshwater fish contain arsenic at levels of 0.05-0.2mg/kg [3] and sometimes at higher concentrations when anthropogenic contamination has occurred. On the other hand, marine animals and algae, because of biotransformation and accumulation, contain high concentrations of arsenic, typically in the range 1-100mg/kg [9,10]. Arsenic is present in water and the earth's crust as inorganic arsenic of different oxidation states (usually +3,+5) while that in marine animals and algae is in organic forms [11,12]. Sea water also contains small quantities of simple organo-arsenic compounds [13].

World annual production of arsenic and arsenic compounds was around 60 000tons/year (in 1975) and arsenic is widely used for the production of alloys and glasses, for agricultural purposes and in the semiconductor industry.

Compounds of arsenic are notorious as poisons but at the same time there are indications that arsenic is an essential element [14-16]. Arsenic toxicity is dependent on its chemical form. Of the inorganic forms of arsenic, arsine is highly toxic, and arsenite is accepted as being more toxic than arsenate [17]. The toxicity or organic arsenic compounds also varies; those of natural origin appear to be non-toxic or of low toxicity, while some synthetic compounds, particularly containing arsenic(III), are very toxic. Generally it would seem that compounds of arsenic(III) are considerably more toxic than those of arsenic(V) [18]. As an example of an apparently beneficial use of arsenic, arsanilic acid and related compounds are known to act as growth promoting factors when fed to poultry [19,20].

Metabolism of inorganic arsenic by marine plants and animals gives rise to a range of organic arsenic species that may be considered as naturally occurring compounds, and these are discussed in the next section. On the other hand, administration of inorganic arsenic compounds to experimental animals produces a small range of simple methylated

metabolites [21,22]. Although such experiments are 'artificial' when compared with the 'natural' metabolism performed by marine organisms, they are necessary for an understanding of the metabolism of arsenic by those persons who are occupationally exposed.

Thus for a complete understanding of the toxicological significance of arsenic as well as any beneficial role, and for understanding the geochemical cycling of this element, it is necessary to know the chemical forms of arsenic that are involved. This article deals with the methods for establishing the speciation of arsenic.

2. CHEMICAL FORMS OF ARSENIC IDENTIFIED IN THE BIOSPHERE

2.1. Marine environment

Much work has been undertaken to identify arsenic compounds in the marine environment. Four arsenic species, arsenic(V), arsenic(III), methylarsonic acid (MMAA), dimethylarsinic acid (DMAA), have been detected in sea water by hydride generation techniques [13,23,24]. It has been shown that algae play a major role in the production of reduced and methylated forms of arsenic in sea water [25]. It has also been demonstrated that microbial demethylation and oxidation of methylated arsenicals occurs in sea water [26,27].

Analysis of marine algae indicated that substantial amounts of arsenic were present in forms other than inorganic or simple methylarsenic compounds [28]. Only small amounts of simple methylated arsenicals were present. Water-soluble and lipid soluble forms of organic arsenic have been detected in algae [29- 31]. Identification of organic arsenic compounds other than simple methylated arsenic acids in algae was made chiefly by ^{1}H NMR spectrometry after isolation of the compounds. The major forms of water-soluble arsenic in marine algae were shown to be 5-dimethylarsinoyl derivatives of 5-deoxyribosides [32-36].

The structure of one such compound was confirmed by X-ray crystallography [37]. A lipid-soluble arsenic compound isolated from the brown kelp *Undaria pinnatifida* was shown to be a phospholipid derivative of a 5-dimethylarsinoyl-5-deoxyriboside [38]. Dimethylarsinoylethanol was isolated from anaerobically incubated brown kelp *Ecklonia radiata* [39]; and was shown to be a decomposition product of the arsinoylribosides, but it has not been confirmed as a naturally occurring compound.

The most frequently reported organoarsenical in marine animals is arsenobetaine. This compound was first identified in the western rock lobster *Panulinus cygnus* by NMR spectroscopy and X-ray crystallography after isolation [40]. Subsequently it was found in a wide range of marine animals including sharks [41,42], American lobster [43], teleost fishes [44-47], crabs [45,48,49], shrimps [50,51], sea cucumber [47,52], cephalopod molluscs [52,53] and several species of gastropod and bivalve molluscs [52,54]. It would seem to be virtually ubiquitous in the many marine animals contributing to the human diet, and in most animals accounts for all or almost all of the arsenic burden. Evidence that extracts of some species of shrimp contain arsenocholine as well as arsenobetaine has been presented [50,55,56]. Arsenocholine was also reported in scallops [57], fishes from a polluted area [58] and in dogfish reference material [59]. However, Shibata and Morita showed that tetramethylarsonium ion and not arsenocholine was present in dog fish reference material [60]. Undoubtedly more work is necessary to confirm the occurrence of arsenocholine in marine organisms.

Some species of fish have been shown by mass fragmentography to contain a small percentage of their arsenic as trimethylarsine oxide [61]. Trimethylarsine oxide was identified in estuary catfish *Cnidoglanis macrocephalus* and school whiting *Sillago bassensis* after oral administration of arsenate and as a natural component of estuary catfish [62]. The methods employed were ^{1}H NMR spectroscopy and electron impact mass spectrometry after isolation of the compound. Arsenobetaine is metabolized to trimethylarsine oxide by bacteria in sediments [63].

Tetramethylarsonium ion, an end product of biomethylation, was identified in the cockle *Meretrix lusoria* by HPLC-ICP and ^1H NMR spectroscopy after isolation [52]. This compound has also been isolated from a clam [64] a sea hare and a sea anemone [65]. It has also been found in the gastropod mollusc *Tectus pyramidis* [66].
Trimethylarsine has been reported in very low levels in some species of deep sea crustaceans [67].
A portion of the arsenic in marine organisms is present in a lipid-soluble form. The oil rich tissues from some marine animals contain lipid-soluble arsenic in addition to arsenobetaine [41,68]. Lipid soluble arsenic in the brown kelp *Undaria pinnatifida* is present as a lecithin-type phospholipid of a 5-dimethylarsinoyl-5-deoxyriboside [38], but it is not certain if arsenic-containing phospholipids are present in marine animals.

2.2. Terrestrial environment

Although the range of arsenic compounds encountered terrestrially is less than that in the marine environment, individual arsenic compounds may present a much greater toxic hazard to persons occupationally or otherwise exposed. Classic work by Challenger [69] established that "Gosio" gas, evolved from damp wallpaper by the action of fungi, was trimethylarsine produced by methylation of inorganic arsenic present in the wallpaper paste. Several people died from chronic inhalation of trimethylarsine from this source. Exposure to inorganic arsenic in high levels by drinking well water has resulted in illness and death [70,71]. Smelter workers exposed to inorganic arsenic through inhalation have been shown to have elevated levels of simple methylated arsenic acids in their urine [72]. Experimental work [73-86] on the administration of inorganic arsenic to experimental animals (mice, rats, rabbits, guinea pigs, hamsters, monkeys) has provided information on the metabolism (particularly the methylation) of accidentally acquired inorganic arsenic.
Arsenic-containing medicines have little place in modern medical practice but at one time were important in the treatment of syphilis and other diseases. However, arsenic containing herbicides (MMAA and DMAA) and veterinary products are still used. It is likely that they are ultimately degraded to inorganic arsenic or volatile arsines by microbial activity [87-90].
In general it would seem that analytical techniques concerned with the terrestrial and atmospheric environments must be applicable to the analysis of a small range of simple arsenic compounds-arsenic(V), arsenic(III), MMAA, DMAA, trimethylarsine oxide (TMAO), and their volatile derivatives, arsine and the mono-, di- and trimethylated arsines.
Arsenic species shown to be present in biological and (or) environmental samples are listed in Table 1.

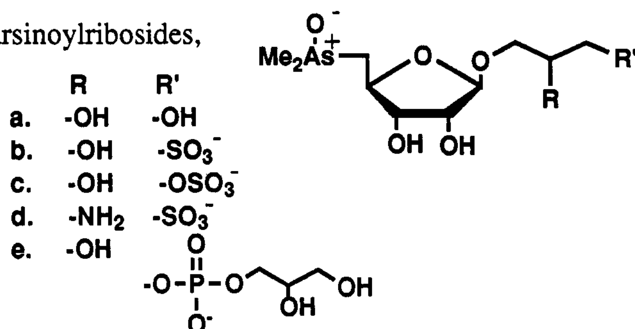
3. SPECIATION METHODS

There are two main approaches to the speciation of environmental arsenic compounds. One is a rigorous procedure that has been employed in natural product chemistry and is appropriate for the identification of compounds of previously unknown structure. Arsenic species are separated from a large quantity of starting material, purified and isolated, and their structures determined by X-ray crystallography, NMR spectroscopy, IR spectroscopy, mass spectrometry, UV-visible spectroscopy and elemental analysis. The method affords an unequivocal identification, but requires a rather large amount of arsenic compound and, usually, much time. This type of approach has been used to provide qualitative data and only limited quantitative data have been given.
The other method is to combine a separation method with selective and sensitive detection methods. A typical method is a chromatographic separation with atomic absorption or emission spectrometric detection. These methods are selective and sensitive if appropriate combinations are made, and are suitable for both quantitative and qualitative analysis if standard arsenic compounds are available. Such methods can also indicate unknown arsenic species by their chromatographic retention data.

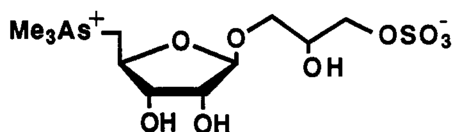
However, it should be noted that there is sometimes a possibility of mis-identification when the only information available on a compound is its retention times in the chromatographic systems. It is likely that the latter methods will be used for speed and convenience once the former techniques have established unequivocally the nature of the arsenic compounds under investigation.

Table 1 Arsenic species encountered in biological or environmental samples

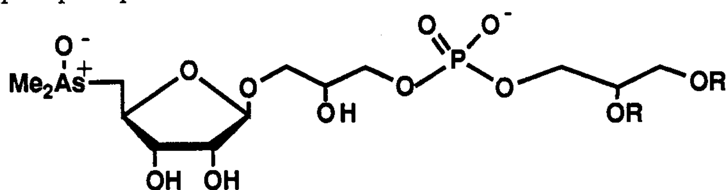
1. arsenic(III), AsO_3^{3-}
2. arsenic(V), AsO_4^{3-}
3. dimethylarsenic acid, DMAA Me_2AsO_2H
4. methylarsonic acid, MMAA $MeAsO_3H_2$
5. trimethylarsine, Me_3As
6. trimethylarsine oxide, TMAO $Me_3As^{+}\text{-}O^{-}$
7. tetramethylarsonium ion, Me_4As^{+}
8. arsenobetaine (trimethylarsonioacetate), $Me_3As^{+}CH_2COO^{-}$
9. arsenocholine (2-trimethylarsonioethanol), $Me_3As^{+}CH_2CH_2OH$
10. dimethylarsinoylethanol, $Me_2As^{+}(O^{-})CH_2CH_2OH$
11. dimethylarsinoylribosides,



12. trimethylarsonioriboside "sulphate ester"



13. dimethylarsinoylribosyl "phospholipid"



R= palmitoyl

3.1. Identification of arsenic compounds

A number of novel arsenic compounds have been discovered in marine plants and animals, and identification of the compounds has been made after their complete isolation. Water-soluble arsenic compounds have been separated and purified by gel permeation chromatography(GPC) (Sephadex LH-20, G-15, G-10 and other resins), ion exchange chromatography (diethylaminoethyl-, carboxymethyl-, and other ion exchange resins), thin layer chromatography (TLC) (silica, cellulose), and high performance liquid chromatography (HPLC) (GPC, silica, reverse phase, ion exchange). Separation of lipid-soluble arsenic compounds has been achieved by GPC (LH-20) and HPLC (GPC, ordinary phase and reverse phase).
X-ray diffraction analysis gives a complete identification. It requires, however, a large amount of sample (usually >10 mg) and a good crystal. NMR spectroscopy (^{1}H, and ^{13}C) usually provides adequate structural information for identification. Several tens of micrograms are usually necessary for a good quality ^{13}C NMR spectrum.
Mass spectrometry (MS) may provide useful data when the amount of the sample available is less than several micrograms. Both field desorption (FD) and fast atom bombardment (FAB) MS have played a role in the identification of highly polar arsenicals. Often these method are most useful in identifying small quantities of known arsenicals, but are also valuable when used in conjunction with NMR spectroscopy for providing structural information on new compounds. The identification of arsenobetaine in shark [41], plaice [46], shrimp [51] and ivory shell [54] was based on the detection of the protonated molecular ion of arsenobetaine at m/z 179 in their FD spectra. Several groups have experienced difficulties in obtaining good-quality FD and FAB mass spectra from biological extracts [55,91]. It has been reported that there are differences between the FD mass spectral behaviour of pure organoarsenicals and those contained in biological extracts. The base peak at m/z 134 of the arsenobetaine standard was reduced to a very low level in extracts of shark, plaice and sole and the m/z 135 ion became the dominant peak. The molecular ion was also very weak or even absent in these samples. It is common in FAB-MS that peaks are masked by matrix ions and ionization of the compounds of interest will also be suppressed by impurities in the extract. It is therefore necessary to purify samples before MS analysis. For unequivocal identification, it may be necessary to use high resolution MS or a tandem mass spectrometric technique which requires more than microgram quantities [92]. In performing quantitative determinations, isotope dilution methods using stable isotope labelled compounds as internal standards will be the only acceptable method.

3.2. Trace speciation methods

3.2.1. *Determination of arsine and methylarsines: hydride generation methods*

Low levels of arsine in, for example, industrial gases or atmospheres have been monitored by the colour change recorded on commercially available arsine-sensitive tape [93] or by chemiluminescent techniques based on room temperature gas phase reaction with ozone. The latter method has a lower limit of detection of $2x10^{-9}$(v/v) arsine in air. Potentially interfering compounds are H_2S, NO, PH_3, SbH_3 [94]. Techniques that use cold traps to concentrate arsine with its subsequent volatilization into instrumentation for selective or non-selective detection have also been reported. Arsines and methylated arsines have been detected and determined by flame [95], electrothermal atomizer [96,97], flame-heated quartz-tube atomizer [98-100], and flame-heated alumina tube atomizer [101,102] atomic absorption spectrometry (AAS), direct current plasma [103], microwave induced plasma (MIP) [104], and inductively coupled plasma (ICP) [105] atomic emission spectrometry. Thermal conductivity [106,107] helium glow [108], MS [109], and electron capture [110] have also been used as detection methods.

When the arsines are specifically generated by the reduction of oxygenated (involatile) arsenic compounds present in biological or other samples, and detection and estimation of the arsines is used as a measure of the involatile arsenic compounds in the original sample , the technique is usually termed a hydride (or arsine) generation method.

An overview of hydride generation techniques has recently been prepared [111]. If each arsine is derived from a sole precursor by the reduction step, the determination of the arsines is a reflection of the original arsenic species present. From this standpoint, the hydride generation method has been used for the elucidation of arsenic species in many samples, particularly those of biological origin.

In the Gutzeit test, a classical arsenic detection method, arsine, measured by the colour change produced by reaction with silver diethyldithiocarbamate, was generated by reduction using zinc and hydrochloric acid [112]. Braman and Foreback reduced arsenic compounds to their corresponding arsines with $NaBH_4$, accumulated them in a liquid nitrogen cold trap and selectively vapourized them into a helium DC-discharge atomic emission spectrometric detector [13]. Differentiation of arsenic(V) and arsenic(III) was achieved by adjusting the pH of the acid reacting with $NaBH_4$. By this method, arsenic(V), arsenic(III), MMAA and DMAA were determined in water, urine, bird eggshells, mollusc shells and limestone at sub-mg/kg concentrations.

Similar hydride generation methods have employed AAS for detection [95,100,113]. Hydride generation with gas chromatography (GC) (packed column separation) and detection by AAS has also been used [102,114]. Cold toluene has been employed as an arsine trap followed by GC separation and MIP atomic emission spectrometric detection [115]. Arsenic(V), arsenic(III), MMAA and DMAA have been determined in animal urine by using a similar heptane trap and GC-MS for separation and detection [109]. It has been shown that HPLC separation-hydride generation can be used to increase the sensitivity when compared with an ordinary nebulizing system for flame AAS, flame atomic fluorescence spectrometry and ICP atomic emission spectrometry [116].

Detailed studies of the hydride generation technique have revealed several problems in the quantitative determination of arsenic(V), arsenic(III), MMAA and DMAA. First, the efficiency of arsine production is dependent on the acidity of the solution and the concentration of $NaBH_4$ [102,103,109,115,117]. Each arsenical has its individual optimum pH for arsine formation and if simultaneous determination is required, it is necessary to use compromise conditions. Second, molecular rearrangement of arsines occurs during reduction. This phenomenon is particularly significant when oxygen is present in the solution [115]. Third, interference, usually reducing the sensitivity, occurs in real samples. Heavy metals and nitrate are known to decrease the signal [103,117], but in many cases the cause of the signal change has not been correctly assigned. Fourth, the purity of the reagents is important. The detection limit for each arsenic compound is, in practice, determined by the blank level for each arsine. Even with these problems, however, hydride generation with AAS, atomic emission or MS detection affords a convenient method for the analysis of arsenic(V), arsenic(III), MMAA, DMAA and TMAO mixtures.

The hydride generation method determines arsine and methylated arsines after the reduction of arsenic(V), arsenic(III), MMAA, DMAA and TMAO; however, it does not identify other arsenic species, i.e., those that do not give rise to volatile derivatives on reduction. In particular, arsenobetaine, the major form of arsenic in marine animals, does not give rise to a methylated arsine on $NaBH_4$ reduction. It has been reported that alkali-digested arsenobetaine gave trimethylarsine by $NaBH_4$ reduction [45] but tetramethylarsonium ion may behave similarly to arsenobetaine. It is also unlikely that the arsenosugars present in marine algae would produce volatile arsine derivatives by $NaBH_4$ reduction [118]. Arsenobetaine and arseno-sugars are apparently the major forms of arsenic in marine organisms and there is a possibility that these forms are released into marine water. Early information on the speciation of arsenic in marine water may need to be reevaluated because these data have been obtained by hydride generation methods.

3.2.2. *High performance liquid chromatography-flame atomic absorption spectrometric detection (HPLC- AAS)*

Atomic absorption spectrometry is an element specific detection method for liquid chromatography as well as gas chromatography. Recent developments in separation techniques using HPLC have made the HPLC-AAS method an effective tool that allows separation and identification of known arsenic species as well as detecting and giving the retention times of unknown arsenic compounds. The combination of HPLC and AAS has been attempted for HPLC-flame AAS and HPLC-electrothermal AAS.

Interfacing a liquid chromatograph to a flame atomic absorption spectrometer requires only a short piece of connecting Teflon tubing [119]. It is recommended that the flow rate from the HPLC be approximately balanced by the nebulizer uptake rate. Generally, flow rates ranging from 2-4ml/min proved to be optimum. When the HPLC flow rate and AAS uptake rate are not balanced (ordinarily the HPLC flow rate is much less), two kinds of device have been proposed. One possibility is to use a three-way connector of which one opening is used for an auxiliary liquid flow to compensate for the deficiency in the HPLC flow rate [120]. The other approach is to use a Teflon funnel micro sampling cup attached directly to the nebulizer. The HPLC effluent is applied dropwise and analyzed without dilution [121].

The sensitivity of flame AAS for arsenic is rather poor. By using the 193.7nm line and an argon/H_2/air flame, the practical detection limit lies around 1µg/ml . When arsenic species are dissolved in an organic solvent, the sensitivity falls. The low sensitivity makes it difficult to apply HPLC-flame AAS to the speciation of arsenic in the low concentrations found in most environmental and biological samples.

There are two major interferences that occur in flame AAS and limit HPLC operational parameters. One is interference by the organic solvents used for reversed phase and ion pair chromatography. The use of an organic solvent changes, in most cases, instrumental response to arsenic by reducing the degree of atomization. It also shifts the baseline of the chromatogram because the transparency at the arsenic absorption line, especially the most prominent line at 193.7nm, is decreased by the introduction of organic solvent to the flame. Thus a gradient of organic solvent causes a serious baseline drift. This problem can often be reduced by using instrumentation that employs background correction.

Usually the HPLC-flame AAS method is suitable for those samples containing a rather high concentration of arsenic (more than 100µg/ml) and are separated by aqueous phase or isocratic organic phase chromatography.

Atomic absorption spectrometry using a long absorption tube and a total consumption burner has been shown to be highly sensitive [122]. The use of such a detector may be another possibility for arsenic speciation.

In addition to the above problems, the sensitivity of flame AAS is dependent on the concentrations of salt and organics contained in the solvents and solute. For the quantitative analysis of real samples, it may be necessary to use the standard addition method by co-injecting known amounts of authentic samples.

Enhancement of sensitivity has been achieved by employing hydride generation methods between HPLC and AAS. Arsenic compounds in the HPLC effluent were reduced to arsines and detected in an electrically heated quartz tube AAS [123]. Determination of arsenic(V), arsenic(III), MMAA, DMAA and *p*-aminophenylarsonate were made by first separating them on a Dionex 3 anion exchange column and then injecting each into an automated arsine generating system coupled to the atomic absorption spectrometer. The method is sensitive but again is applicable only for those molecules that produce volatile arsines on $NaBH_4$ reduction. Similar determinations have been carried out using flame heated tubes in the atomic absorption spectrometer [124,125].

3.2.3. *High performance liquid chromatography-electrothermal atomic absorption spectrometry (HPLC- ETAAS)*

Electrothermal atomic absorption spectrometry (ETAAS), usually employing a graphite furnace or atomizer, has improved sensitivity by up to two orders of magnitude when compared to flame AAS.

Coupling of ETAAS detectors to HPLC involves a number of problems because of the stepwise operational characteristics of the commercially available atomizers and because only a small volume at a time can be injected into them. Two interfacing methods have been proposed [126-128]. First, on-stream sampling in which 10-50µl of the effluent solution is sampled periodically (e.g. each 40s) from the effluent stream. Second, off-stream sampling in which the automatic sampler of the ETAAS spectrometer is used as a fraction collector. In the on-line sampling method, the resolution of HPLC is sacrificed by the relatively low frequency of sampling caused by the heating cycles of the atomizers. With off-line sampling, the resolution of HPLC is lost to a lesser extent but the total time for analysis increases.

The sensitivity of ETAAS and the general response of the instruments are dependent on the characteristics of the graphite and the other parts of the atomizer system. The cuvettes, for instance, tend to deteriorate with use and reduce the sensitivity. A new cuvette does not necessarily produce the same response as one subjected to a number of analysis cycles. This behaviour necessitates the frequent use of standards.

Although HPLC separates matrix components from arsenic species, there is still interference inherent in performing the ETAAS analysis. The interferences in general lead to a reduction in the observed atomic absorption signal per nanogram of element in the sample. Two interferences which are pertinent to HPLC- ETAAS analysis of organo arsenic compounds are the incomplete decomposition of molecular species to the elements of interest, and the incomplete atomization in the presence of salt and (or) carbonaceous material. The former reaction is a problem with compounds which are thermally stable and can be vapourized without decomposition.

Different arsenic compounds produce different ETAAS responses for the same quantity of arsenic; as much as a two fold difference in the intrinsic ETAAS sensitivity was observed between DMAA and MMAA as well as between their sodium salts [129]. Less volatile species gave a more intense signal than those of greater volatility.

Atomization processes are likely to be different for each compound and a greater loss of the more volatile derivatives from the cuvette during atomization is a probable cause of the observed behaviour. It is also likely that significant amounts of organic materials eluted at the same positions as the arsenic peaks will reduce the atomic absorption signals. In this case, it may be necessary to use the standard addition method by co-injecting known amounts of authentic samples.

The addition of nickel to the solution is reported to eliminate the species dependency of the signal [130]. Nickel also prevents the loss of As during the ashing stage and allows a higher ashing temperature. In an ion exchange separation, nickel treatment of the solution prior to ETAAS allowed an ashing temperature of 1000°C [131].

3.2.4. *High performance liquid chromatography-inductively coupled plasma-atomic emission spectrometry (HPLC-ICP-AES)*

The direct coupling of HPLC to an ICP-AES detector offered the possibility of continuous monitoring of arsenic. The technique has been applied to the separation of selected arsenic compounds, using ion-exchange and GPC techniques [132].

The connection of a HPLC and an ICP spectrometer is simple and employs a short piece of Teflon tubing between the two instruments [133,134]. Because the ICP-AES nebulizer uptake rate is 1-2ml/min which is close to the conventional HPLC flow rate, it is usually not necessary to adjust the flow rate. When a micro-column is used for HPLC, addition of an auxiliary solvent or using a special cross-flow nebulizer has been tried [135,136]. Arsenic is monitored at the wavelength of the prominent arsenic lines; the emission line at 189.0nm is the strongest emission line but can only be used with vacuum or purge type spectrometers. The emission line at 193.7nm has greater sensitivity than the 197.2nm or 228.8nm lines but is affected by the carbon content in the solution because of the carbon emission line at 193.1nm. The 228.8nm line [137] is the least sensitive but is not affected by the carbon interference. The 197.2nm line is intermediate both in terms of sensitivity and carbon interference. Thus, the 193.7nm line or the 197.2nm line is used for aqueous phase ion exchange chromatographic separation and the 228.8nm or the 197.2nm line are used for reversed phase ion pair chromatography that employs organic solvents [137].

The response of the ICP-AES might be expected to be a constant function of the amount of arsenic injected, irrespective of the compound, because the ICP temperature is sufficiently high to facilitate complete atomization. Indeed, it has been reported that the relative sensitivities (in terms of peak area response) for four arsenicals were almost the same (0.99-1.02) in a separation by ion-exchange chromatography [132]; and very similar (0.96-1.02) for four arsenicals separated by ion-pair, reversed phase chromatography [138]. However in one study that used gradient elution ion-exchange chromatography, it was reported that the peak area response depended upon the nature of the compound. The latter dependency may arise from the ICP conditions [139].

Studies [132,137] have demonstrated that the detection limit depended not on the peak area, but on the signal height; hence, it showed a chemical form dependency. Taking the limit at S/N=2, it was 30, 19, 41, and 30ng, respectively, for arsenic(III), MMAA, DMAA, and arsenic(V) in ion exchange chromatography [132], and 150 and 75ng for arsenic(III) and arsenic(V) in reversed phase ion pair chromatography [137].

Possibly further nebulizer modifications will reduce the peak broadening and improve the sensitivity of this method. A glass frit nebulizer [140], a direct injection nebulizer [138], and a thermospray interface [141] have been used. However, only marginal improvements seem to have been achieved by the introduction of these methods.

The photomultiplier output of the As emission line is affected by emission from other elements. Selectivity may be defined as the number of moles of the interfering element required to produce the same emission as a mole of arsenic. Selectivity depends on the wavelength of the As emission line, the resolution of the spectrometer, the ICP operational parameters, the major solutes in the HPLC solvent, etc. Examples of the reported selectivity of the As 193.7nm line were: more than 10^4 for Ca, Cd, Cu, Hg, Zn, B, P, Se, and K ; 10^3-10^4 for Co, Mn, Fe, Si, Ni, Cr and Sn ; 500 for Mo; 300 for Pb; 195 for Al and 51 for Ti [132]. With the As 228.8nm line, Cd interference (228.8nm) may be a serious problem if Cd species are present in the effluent [137]. Interference by sodium chloride has been considered in terms of a change in background emission [142]. Appropriate interference correctors can probably reduce these spectrometric interferences.

Chromatographic separation of twelve arsenic compounds included in Table 1 using four different chromatographic conditions including anion-exchange, cation-exchange, and GPC has been recently reported [52]. With the use of HPLC-AAS or HPLC-ICP, the only information obtained for each compound is its retention times in the chromatographic systems. Examination of retention times on different columns under a variety of conditions, and comparison with authentic samples is therefore necessary to improve the rigour of the identification.

3.2.5. *Other methods*

Several papers have described the speciation of arsenic in various matrices. Methods used include TLC-AAS [44,143], paper chromatography-AAS [144], paper electrophoresis-AAS [145], ion exchange column chromatography-AAS for the separation and detection of arsenic(III), arsenic(V), MMAA, DMAA [146,147], GC with flame ionization detection (FID) and GC-MS of methylthioglycolate derivatives of MMAA and DMAA [148]. GC-MIP and GC alkali flame ionization for the estimation of the ethylene glycol derivative of MMAA [149], GC-FID and flame spectrometric detection of the trimethyl-silyl [150] and GC-electron capture detection of the diethyldithiocarbamate complex of MMAA and DMAA [150]; solvent extraction followed by detection by AAS [151,152], and polarography/voltammetry to discriminate arsenic(III) and arsenic(V) [153,154]. These methods, however, are not likely to possess significant advantages over hydride generation-GC, HPLC-AAS and HPLC-ICP.

Inductively coupled plasma-mass spectrometry ICP-MS has extremely high sensitivity and, when coupled with a HPLC, is likely to be a suitable method for samples containing low concentrations of arsenic compounds [59,60,155]. HPLC-MS, especially plasma spray interface mass spectrometry, may also prove to be a valuable analytical method. Application of these methods to real samples will verify their validity.

3.2.6. *Methods for the determination of arsenobetaine in marine food samples*

Although there are many toxic compounds of arsenic, it has been usually accepted, mainly because of lack of evidence to the contrary, that the arsenicals in traditionally eaten marine-derived foodstuffs do not pose any hazard to human health. The major form of arsenic in fish and crustaceans [40-54] is arsenobetaine, and this has been shown to be non-toxic [86,156- 159]. However, it might be considered necessary, for reasons of toxicological reassurance, that the concentrations of arsenobetaine and other arsenicals in foodstuffs of marine origin be able to be determined by methods which can be routinely applied.

Most studies, as outlined elsewhere in this paper, have been qualitative rather than quantitative when dealing with real samples, or at least have employed methods unsuitable for routine analysis. Quantitative determination in real samples requires the establishment of efficient extraction processes, good recovery in clean-up procedures, and precision and accuracy controls. Few papers have addressed these problems.

Arsenobetaine is very soluble in water and methanol. It is soluble in ethanol but only slightly soluble in acetone and chloroform. Extraction from tissue homogenates has been usually performed using methanol [57]. Chloroform does not extract arsenobetaine from the fish homogenate. Chloroform/methanol mixtures extract arsenobetaine depending on the methanol concentration. Recovery of 101+4% was reported for the chloroform / methanol (1:2) extraction of freeze-dried fish muscle spiked with arsenobetaine [59]. Recoveries of 80-83% for spiked samples of fresh fish after a 24hr methanol Soxhlex extraction and subsequent clean-up process were reported [56]. For routine analysis of arsenobetaine in marine samples, HPLC-ICP-AES would appear to be a good choice in terms of sensitivity, selectivity and ease of operation [48,132,160]. When the sensitivity is not sufficient, HPLC-ICP-MS may be used.

How ever these instruments are expensive and not available in many laboratories. Consequently the use of AAS, in particular ETAAS, as an element specific detector needs to be considered. Preliminary chromatographic separation of arsenobetaine from other arsenic species is, of course, necessary before spectroscopic estimation. Hydride generation AAS has sufficient sensitivity for most marine food samples, but again chromatographic separation of arsenobetaine from other arsenicals must be achieved before reliable estimation can be made. An additional problem is the alkaline digestion necessary to render arsenobetaine amenable to reduction (see earlier in this paper). Only limited information is available on the concentration of arsenic species in environmental and biological certified reference materials [59,60].

3.2.7. *Methods for the determination of arsenicals in soil samples*

The organic arsenicals MMAA and DMAA have been extensively used as herbicides and analytical methods have been developed for the quantitative determination of these species, together with arsenate and arsenite, in soil samples. Although a solvent extraction method combined with column chromatography has been employed for the separation of the four species [161], Hydride generation [162,163] or HPLC [129,164] is usually preferred. Estimation of the separated species has been carried out by DC helium emission spectrometry [162] but normally ETAAS was the method employed [129,161,163,164].

However there are problems with the extraction and clean-up of the arsenic residues before the analytical process can be used. One study [164] extracted soil samples with 2M aqueous ammonia, cleaned them on a carbon-celite column before concentration for HPLC-ETAAS and claimed recoveries of DMAA 90%, MMAA 83%, arsenic(V) 56%, and arsenic(III) 64%. The limit of detection in soil was 0.5ppm. Another study [162] using 0.03M NH_4F and 0.025M HCl for extraction found inorganic arsenic species much more strongly sorbed, with consequent lower recoveries, to clay soils than to loams (recoveries were about 75% for loams and about 20% for clays). The differences between soil types were less for the organic herbicides with 46-63% of MMAA and 76-96% of DMAA

recovered. The authors also attempted direct analysis of unextracted soils that had been spiked with the four arsenicals. They found a considerable loss of precision but offered it as a rapid semiquantitative method.

4. CONCLUSIONS

Speciation studies have been carried out by two different approaches, one to identify unknown arsenic compounds by rigorous methods, and the other to identify compounds in experimental samples by comparison with standard compounds using chromatographic separation coupled with element-specific detectors. The continued application and development of both approaches is necessary for complete speciation of environmental and other samples; i.e. the identification of previously unknown species and the routine estimation of known compounds. The latter will undoubtedly require increased use of appropriate certified reference materials. Methods chosen for the routine analysis of arsenic species in environmental samples will depend on a number of factors such as cost, instrument availability, reasons for undertaking the analyses, expected concentrations, species to be determined etc. It follows that the choice of instrumental method for a particular task or range of tasks will require some degree of critical assessment by the potential user, and hard and fast recommendations are likely to be unhelpful.

Although a major advantage of element-specific spectroscopic detectors lies in the general lack of interference from the sample matrix, sample preparation is the area that requires most attention as it still represents the main source of problems encountered in speciation work. These problems include matrix interference, incomplete recovery, and analyte instability. Few papers have referred to the recovery of arsenic species from real samples.

Important problems of arsenic speciation remain unresolved because of the low concentrations of arsenic compounds involved. These include the chemical forms of arsenic in terrestrial organisms, including man. Such compounds may have metabolic or nutritional importance. The continuing development of atomic spectroscopic detectors, including ICP-MS will undoubtedly, through improved sensitivity, selectivity and precision, bring such problems within the scope of the analytical chemist.

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