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The Speciation of Arsenic Compounds

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It is known that arsenic demonstrates species-dependent toxicity. Quantification of elemental species in a sample, rather than determining total element levels alone, provides information that can be utilised in assessing toxicity, bioavailability and potential effects on the environment. High performance liquid chromatography (HPLC) coupled with inductively coupled plasma mass spectrometry (ICP–MS) has proved an extremely efficient tool for identification and determination of arsenic species providing limits of detection at $\mu g\ L^{-1}$ levels. Low-pressure ion exchange chromatography using mini-columns can provide a simple, yet effective, method for rapid screening of samples. Inorganic forms of arsenic can be separated from organic forms within 3 min with limits of detection at 1.6 and 1.8 $\mu g\ kg^{-1}$, respectively. The increasing use of electrospray techniques to provide structural information has enhanced the ability to distinguish with confidence previously unidentified species.

Toksyczność arsenu zależy od postaci, w której występuje. Dlatego też toksyczność, dostępność biologiczną, czy też potencjalny wpływ na środowisko można lepiej ocenić oznaczając w próbce poszczególne formy arsenu, a nie tylko jego całkowitą zawartość. Wykazano, że bardzo dobrą techniką do identyfikacji i oznaczania związków arsenu, zapewniającą granice detekcji na poziomie $\mu g\ L^{-1}$, jest wysokosprawna chromatografia cieczowa połączona z plazmą indukcyjnie sprzężoną i spektrometrem mas. Prostą i efektywną metodą szybkiego klasyfikowania próbek jest niskociśnieniowa chromatografia jonowymienna z zastosowaniem mini-kolumn. Nieorganiczne postacie arsenu można oddzielić od postaci organicznych w ciągu trzech minut, uzyskując granice detekcji odpowiednio 1.6 i 1.8 $\mu g\ kg^{-1}$. Wzrost stosowania technik elektrorozpylania (dających możliwość badania struktury) pozwolił rozróżnić, z dużą dozą pewności, formy uprzednio trudne do zidentyfikowania.

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The concept of trace metal or elemental speciation has become an area of increasing interest in analytical chemistry. The significance of speciation in understanding the toxicological, nutritional and biochemical impact of any element on a biological system depends on the chemical forms present. The concentrations of individual elemental species are more relevant in the setting of environmental and biological standards than are total elemental concentrations. Qualitative and quantitative separation schemes need to be co-ordinated together with total element concentration measurements to obtain the most meaningful analyses.

Arsenic is an element that has been the subject of many studies as it is a known toxin. It is a ubiquitous element in the environment having been introduced *via* natural and anthropogenic routes. Arsenic readily undergoes conversions mediated by microorganisms, plants and animals, which explains the wide variety of arsenic species that have been identified. Its toxicity can vary by several orders of magnitude depending on the chemical form.

CHEMISTRY AND BIOGEOCHEMICAL CYCLING OF ARSENIC

Arsenic is a metalloid element and is monoisotopic at a mass of 74.92 g. Arsenic can exhibit differing oxidation states, the most common ones being III and V. It has a first ionisation potential of 947 kJ mol^{-1}.

Arsenic can be found in the environment in organic and inorganic forms. Inorganic forms of arsenic most commonly present are the arsenate [As(V)] and arsenite [As(III)] acids. Arsenous acid and arsenic acid are water soluble and, depending on the pH, can be present in a number of ionic forms:

Arsenous acid

$HAsO_2 + H_2O \leftrightarrow H_3O^+ + AsO_2^-$ pK_a 9.23

Arsenic acid

$H_3AsO_4 + H_2O \leftrightarrow H_3O^+ + H_2AsO_4^-$ pK_a 2.20

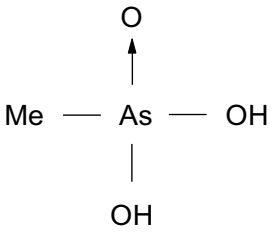
$H_2AsO_4^- + H_2O \leftrightarrow H_3O^+ + HAsO_4^{2-}$ pK_a 6.97

$HAsO_4^{2-} + H_2O \leftrightarrow H_3O^+ + AsO_4^{3-}$ pK_a 11.53

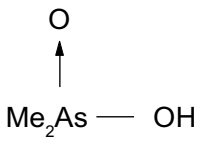
Inorganic arsenic is dominant in aquatic systems and is assimilated into many organisms where biotransformations give rise to the variety of arsenical species seen. Research has been abundant in this field and many arsenic compounds have been identified and mechanistic pathways for their formation proposed [1,2]. However, work continues to enable a more complete understanding of arsenic biogeochemical cycling.

The most common organic arsenic compounds are monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), tetramethylarsonium ion (TeMA), arsenocholine (AsC), arsenobetaine (AsBet) and arsenosugars.

Structures of these organoarsenic species are as follows:

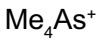


MMA

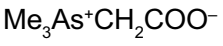


DMA

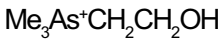
TMAO



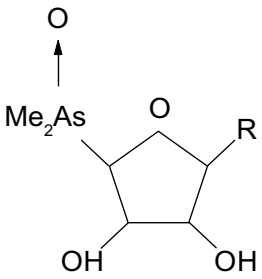
TeMA



AsBet



AsC



Arsenosugars

Biogeochemical cycling of arsenic plays a crucial role in the bioavailability and toxicity of species present in the environment. Arsenic cycles within the environment are not yet fully understood although much work has been achieved towards this goal. As concentrations of arsenic in terrestrial plant foods are generally regarded as being significantly lower than those found in marine organisms much of the work on the biogeochemical cycling of arsenic has focussed on aquatic environments. It is known that dissolved arsenic may be scavenged from the water column by surface-active suspended particulate matter (SPM) and by phytoplankton [3] with removal by phytoplankton being the dominant process [4]. Arsenic may also undergo chemical transformations and remobilization due to redox and methylation reactions within sediments [5]. Phytoplankton can metabolize inorganic arsenic As(V) to methylated spe-

cies and more complex molecules, *i.e.* arsenosugars [6]. It has now been demonstrated that there are seasonal fluxes in the levels of inorganic arsenic species compared to that of methylated arsenic species attributable to variations in the amount of phytoplankton activity [3,4,7,8]. The presence of methylated species is also associated with water temperatures greater than 12°C, possibly due to microbial degradation of planktonic tissue [9]. This evidence suggests that marine organisms are capable of transforming toxic inorganic forms of arsenic to less toxic forms by metabolic methylation. Classic studies by Challenger [1] on microbial methylation of arsenic still provide the basis for current understanding of these processes. Arsenosugars are the major source of arsenic in marine algae whereas arsenobetaine is the predominant form found in marine animals. The pathway for the formation of arsenobetaine in marine animals remains unsubstantiated although it is hypothesized that it may originate from arsenocholine, the pathway for which is also unknown, or that it is selectively accumulated from water [2].

Inorganic forms of arsenic, As(III) and As(V), are the most toxic. Organic species display decreasing toxicity with increasing derivatisation. The LD_{50} values in rats, mg kg^{-1}, in decreasing levels of toxicity has been given as: arsenite, 1.5; arsenate, 5.0; MMA, 50; DMA, 600 [10]. Other work suggests that the more highly methylated the compound the higher the toxicity: LD_{50} TeMA, 890 mg kg^{-1}; DMA, 1200 mg kg^{-1}; MMA, 1800 mg kg^{-1} [11]. Overall, the LD_{50} levels suggest much lower toxicity for organic species than inorganic species. Arsenobetaine and arsenocholine have been shown to be essentially non-toxic with $LD_{50} > 10$ g kg^{-1} [12]. It is also thought that arsenosugars found in seaweed and algae are principally harmless [13].

Human exposure to the risks of arsenic has become of considerable interest because of the greater understanding of its speciation, bioconcentration and biomagnification. The presence of arsenic in humans may be from food intake, particularly seafood, from drinking water or from occupational exposure in industries making such products as glass, wallpaper and non-ferrous metals [14]. The absorption of arsenic depends on the species of analyte present. Greater than 95% of inorganic arsenic is absorbed, approximately 75% of MMA and DMA is absorbed whilst arsenobetaine is not absorbed by the body at all [15]. Seafood is known to be the most significant source of arsenic in the diet [16]. Concentrations in marine animals generally lie in the range of 10–500 mg kg^{-1} but have been known to exceed 1000 mg kg^{-1} for organisms living in contaminated areas [12]. Excess exposure to arsenic can give rise to mutagenic, teratogenic and carcinogenic effects [17]. Fortunately, arsenobetaine has been reported as the most commonly found organoarsenic compound in marine animals, fish and crustaceans [18] which as noted above is essentially non-toxic. Arsenobetaine can account for up to 100% of the arsenic present in fish [19]. Guidelines from the World Health Organisation (WHO) suggest a provisional maximum tolerable daily intake of inorganic arsenic at 2 μg kg^{-1} of body weight with a 20% allocation of this to

drinking water. On this proviso, levels in drinking water should not exceed 10 μg L^{-1} [20]. A recent incident in Bangladesh where tube-wells were installed to provide clean water in order to reduce the incidence of gastrointestinal disease led to the release of arsenic into the drinking water [21]. Levels of total arsenic were found between 600–1100 μg L^{-1} [22]. Approximately 2/3rd of the population have been affected to some degree by this poiso-ning [23]. Included in recommendations from the World Health Organisation following this disaster is that all drinking water must be tested for arsenic prior to consumption [22]. Field deployable electrochemical techniques have been used with some success for this purpose [25].

Human urine is a suitable biological specimen for monitoring recent exposure to arsenic because it is the main excretory pathway and has relatively fast excretion rates. It is thought that there is no interconversion between toxic forms (As(III), As(V), DMA and MMA) and non-toxic forms (arsenobetaine and arsenocholine) *in vivo* and, therefore, it is possible to reveal the source of arsenic, *i.e.* from occupational exposure (toxic species) or from food (non-toxic species) [15]. However, recent studies [26,27] suggest that arsenosugars (mainly dimethylarsinoylribosides), although themselves being essentially non-toxic, can be metabolised within humans to the more toxic organic compound, DMA.

APPROACHES TO SPECIATION

Analytical capability to detect arsenic species relies on the ability of the instrumental method to provide not only species selectivity but also precise and accurate data. To accomplish this it is necessary to achieve either good separation of the species or selective detection and to be certain of the identification of elemental species as well as to minimize interferences. Standards for the identification and determination of species can be employed together with certified reference material for method validation. However, the ability to obtain structural information from detection schemes is of increasing importance to ensure no misidentification of the growing number of compounds that have been extracted from samples and reported in the literature [28].

Most analytical methods used for arsenic speciation are based upon coupling of instrumental techniques for separation followed by sensitive, element-specific detection. Numerous instrumental methods have been developed and can be used according to requirements. As(III) and As(V) are the most frequently determined species [29,30], due to their high toxicity, with organic arsenic species requiring a separation step and/or pre-concentration depending on the sensitivity of the detector [31]. A review by Burguera and Burguera [32] gives a comprehensive account of the various instrumental techniques most commonly used, *i.e.* spectrophotometric and electroanalytical methods, hydride generation atomic absorption spectrometry (HG–AAS), in-

ductively coupled plasma mass spectrometry (ICP–MS) and atomic emission spectrometry (ICP–AES), amongst others. Comparison of techniques indicate that HPLC–HG–ICP–MS can be conveniently used for multi-element studies whereas HPLC–HG–FAAS can be used for more routine studies [33]. The advantage of AAS is that it is simple to use and well un-derstood. However, current literature suggests that high performance liquid chromatography (HPLC) coupled with ICP–MS is one of the more commonly employed techni-ques for speciation, identification and determination, because of the separation achievable by HPLC and the inherent sensitivity and selectivity of ICP–MS. The main separation techniques based upon HPLC for arsenic species are ion exchange and reversed-phase and both of these techniques are widely documented [19,34]. As previously mentioned, structural information on species extracted from samples is necessary to improve the scope achievable by analytical techniques. Electrospray mass spectrome-try (ESMS) is rapidly becoming the instrument of choice in this area of chemistry as it provides both quantitative and qualitative information [33].

As with all analytical instruments, interferences need to be considered in order to obtain the best possible results. Limits of detection for HPLC–ICP–MS are often quoted to be at μg L^{-1} values [36], but in the case of arsenic major spectroscopic and non-spectroscopic interferences are encountered. Many samples under investigation for the presence of arsenic species are derived from the marine environment, hence, the most obvious spectroscopic interferent is that caused by the formation of $^{40}Ar\ ^{35}Cl^{+}$ in the plasma [37]. Signal interference is strongly related to the chloride concentration in the sample matrix [38]. As arsenic is monoisotopic it is impossible to avoid this isobaric overlap with conventional quadrupole mass analyzers. Research in this field has succeeded in reducing the interference of Cl^{-} in a sample matrix by the addition of nitrogen into the plasma [39–41]. Another way successfully employed to overcome this interference has been the use of an anion exchange column that will retain the Cl^{-} thereby preventing it from entering the plasma [42]. The use of HG methods for the determination of arsenic species coupled with ion chromatography–ICP–MS can also eliminate the Cl^{-} interference because arsenic compounds are converted to their volatile arsines which can then be separated from the liquid eluent and introduced in the ICP–MS without matrix interference. One drawback of this technique is that arsenobetaine and arsenosugars do not form volatile hydrides and, therefore, the destruction of the organic part of the molecule is required prior to HG. However, as inorganic arsenic poses the greatest risk to the environment HG can be a useful screening method, particularly when automated, providing limits of detection at 0.79 μg L^{-1}. The major non-spectroscopic interference arises from the high first ionization potential of arsenic. Instrumental optimization of all working parameters, *i.e.* nebuliser gas flow-rates and radio frequency power, will ensure the best sensitivity achievable to alleviate this problem.

In the determination of arsenic species sample pre-treatment needs careful consideration. The selection must consider the efficiency of the procedure and any effects there may be on preserving or changing of original species. Common extraction procedures have relied on methanol-water methods [33,41–43], and enzymatic digestions [19,44]. Recoveries of up to 100% can be obtained with no obvious species interconversion or degradation. For total element analysis microwave digestions can be employed as described by Branch *et al* [45].

Further to arsenic speciation by HPLC–ICP–MS, a technique based upon ion exchange liquid chromatography with low-pressure mini-columns has been developed at the University of Plymouth. Preliminary findings suggested that separation of organic from inorganic or assumed non-toxic from toxic arsenic species can be achieved in a simple yet effective way. To arrive at the separation of organic and inorganic arsenic using mini-column technology, many experimental conditions were considered. The counter ion (phthalate, sulfate and phosphate) and its concentration (5 mmol L^{-1}–0.1 mol L^{-1}) were examined, pH conditions ranging from 4–10, both Benson AX10 (25 × 3 mm) and Hamilton PRP X100 (50 × 3 mm) (both resins being strong anion exchange resins) micro-columns were assessed for suitability and eluent flow-rates were varied (1–2 mL min^{-1}). Separation of As was achieved using Benson AX10 anion exchange resin using a gradient elution of MilliQ water followed by 0.1 mol L^{-1} potassium hydrogen phthalate (Table 1).

Table 1. Chromatographic parameters for mini-column separation of arsenic species

First mini-column system for arsenic separation
Resin: Benson AX10 , 7–10μm particle size Column diameters: 25 mm × 3 mm Mobile phase: MilliQ water (2 min), 0.1 mol L^{-1} potassium hydrogen phthalate (5 min) pH 10.2 Flow rate: 1.4 mL min^{-1}
Second mini-column system for arsenic separation
Resin: Hamilton PRPx100 , 12–20 μm particle size Column diameters: 100 mm × 3 mm Mobile phase: 10 mmol L^{-1} potassium sulfate, pH 10.2 (5 min) Flow-rate: 1.0 mL min^{-1}

Using the Hamilton mini-column, separation of organic (AsBet, DMA) from inorganic arsenic species As(III), As(V) was achieved using an isocratic elution with 10 mmol K_2SO_4 at pH 10.2 (see Tab. 1). Hydrogen peroxide (30% v/v, 0.25 mL in 25 mL) was added to arsenic standards in order to oxidize As(III) to As(V) allowing the inorganic components — to elute together. The presence of the H_2O_2 did not appear to alter any of the other standards tested although further tests involving real samples is necessary to fully assess the impact of the H_2O_2. A typical chromatogram of the separation can be seen in Figure 1. The use of isocratic conditions enables rapid

sampling within 3 min and no column equilibration needs to be employed between injections. Limits of detection obtained for inorganic arsenic and organic arsenic were 1.6 and 1.8 μg kg^{-1}, respectively.

The optimum experimental conditions arrived at for both column systems under investigation provided the best separation whilst keeping the total solids in the mobile phase to a minimum, to reduce interferences caused by salt deposition in the ICP–MS torch injector or sampling cone orifice.

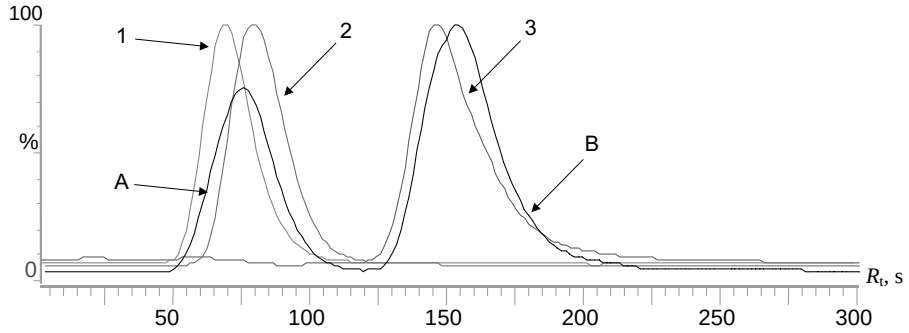


Figure 1. Separation using Hamilton PRPX100 resin of arsenic species, individually: 1 = arsenobetaine, 2 = dimethyl arsonic acid, 3 = arsenate and arsenite; simultaneously: A = organic arsenic, B = inorganic arsenic, demonstrating species retention times (concentrations of 100 ppb)

FUTURE WORK IN THE FIELD OF ARSENIC SPECIATION

Technology is constantly improving leading to an increasing number of arsenic species that can be identified and determined along with the mechanistic pathways involved. This work is essential in promoting understanding of the environment and how to protect it from the potential threat of pollution. Continued research with HPLC–ICP–MS and ESMS is highly recommended.

However, when monitoring pollution hazards it is not always necessary to carry out a full speciation analysis. Arsenic lends itself to the separation of inorganic from organic forms, *i.e.* assumed toxic from non-toxic species. For this type of work HPLC–ICP–MS demonstrates two main disadvantages; 1) it is very expensive to operate, and 2) analysis times can be exceedingly long. To provide a cost-effective screening protocol for regulatory agencies fast, efficient and simple to operate separation techniques need to be developed. This would then allow for a greater number of samples to be analyzed in the same amount of time thereby reducing the running costs of the ICP–MS.

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