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PM Yehl

H Gurleyuk

JF Tyson

PC Uden

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Peter M. Yehl,† Hakan Gurleyuk,‡ Julian F. Tyson* and Peter C. Uden

Department of Chemistry, University of Massachusetts, 701 Lederle Graduate Research Tower, 710 North Pleasant St., Amherst, MA 01003-9306, USA.
E-mail: Tyson@chem.umass.edu

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Arsenic-containing species were extracted from soil and sediment SRM by a mixture (1 + 1) of acetone and hydrochloric acid (10% v/v) in a sealed vessel in a microwave oven during heating to 160 °C at pressures up to 1150 kPa (160 psi). Following separation by anion-exchange HPLC those species which gave a volatile derivative on reaction with borohydride in acid were detected by plasma-source mass spectrometry. The procedure was used to determine the monomethyl arsonate species in SRM 2704 Buffalo River sediment (0.30 ppm), SRM 1944 New York–New Jersey waterway sediment (0.23 ppm) and in SRM 2710 highly elevated Montana soil (1.03 ppm). The method was developed by investigating the recovery of dimethylarsinate added to Buffalo River sediment as a function of various experimental parameters, including the composition of the solvent. For 1 + 1 mixtures of acetone and 5% HCl, methanol and 5% HCl, and isopropanol and 5% HCl, recoveries ranged from 91% to 112%. Similar recoveries were obtained for ultrasound-assisted extractions with the same solvents. The chromatographic eluent was not directly introduced into the mass spectrometer as, compared to the post-column hydride generation procedure, the sensitivity was too low for reliable quantitative measurements, although the chromatographic resolution was better. Problems with signal pulsations were overcome by incorporating pulse dampers into the reagent delivery lines.

Introduction

Biological methylation of arsenic in fungal cultures has been understood for many years.[1] Methylation of arsenic by bacteria is known to occur through a metabolic reaction which uses methylcobalamin (the methyl transfer compound) and adenosine triphosphate (the energy source) in controlled anaerobic climates,[2] and in lake sediments.[3] The rate of production of monomethyl arsonic acid by arsenic-tolerant anaerobic bacteria has been studied.[4]

Naturally existing methylated arsenic species are probably the result of a biological transformation of inorganic arsenic. Biological methylation of antimony in a soil medium,[5] and of selenium,[6] tin,[7] tellurium,[8] mercury,[9] and lead,[10,11] predominantly in anaerobic cultures, have been demonstrated. It has been shown that antimony is reduced prior to methylation.[5] It can be speculated that methylated forms of arsenic exist in nature, and are probably the result of biological transformation,[12] but to date very few studies have found methylated arsenic in either soils or sediments. A recent study has shown that biological conversion of arsenic to various methylated forms takes place by the fungus *Laccaria amethystina* or its associated bacteria in a largely anaerobic environment.[13] This work demonstrated that such a conversion is not restricted to marine biota.

Arsenic speciation in nature is important from a number of perspectives. Different forms of arsenic have vastly different toxicity to humans due to human bioavailability. For example, inorganic arsenic [As(III) and As(V)] are carcinogenic, and can cause neurological, cardiovascular, and hematological disorders,[14] while monomethyl arsonic acid (MMA) is believed to be far less toxic.[15] The long-term effects of dimethyl arsinic acid (DMA) are not fully understood.[16] Still other forms (arsenobetaine, arsenocholine) have been deemed essentially inert, and are excreted readily by humans with little or no absorption.[17] Arsenic compounds such as arsenic metal oxides and arseno-phosphates that are not readily water soluble have been shown to be less toxic than water soluble compounds because they are less bioavailable.[18,19]

The chemical speciation of arsenic affects its environmental mobility. Inorganic As(V) is relatively sedentary in soil, and can be adsorbed onto clays or precipitated with sulfur, iron and aluminum.[20] Methylated As(V) is less likely to precipitate and therefore is mobilized when it comes into contact with water. Although As(III) can be volatilized more readily than As(V), and there is a wide range in the degree of volatility between methylated As(III) species.

Elemental speciation in a soil matrix requires the extraction and separation of the species without changing the chemical form. For the trace concentration speciation of arsenic, the separation must be coupled with a detection technique having low detection limits, such as ICP-MS, ICP-OES or AAS, capable of monitoring transient chromatographic signals. The recent literature contains many papers describing arsenic speciation in a number of matrices. Some of the procedures that were first employed to quantify arsenic species are given in Table 1.

† Present address: Merck Research Laboratories, P.O. Box 2000, Building RY-818-B215, Rahway, NJ 07065, USA.
‡ Present address: Frontier Geosciences Inc., 414 Pontius Avenue N., Seattle, WA 98109, USA.

Arsenic extraction and speciation have been attempted for a number of matrices including urine,[23,34,41] marine biological tissues,[23,35,40,42–55] mushrooms,[12] and soils,[28–31,56,57] but to date there has been no work in which individual methylated arsenic species in soil or sediment SRM were quantified. Analytical methodology for the extraction, recovery and determination of arsenic species has been reviewed.[58] Examples of the extraction of organometallic compounds from various environmental matrices are given in Table 2. Methylated arsenic forms were not detected in water extracts.[30] The use of a solvent capable of ion-exchange or forming an ion-pair may be desirable when extracting ionic species such as MMA or DMA, particularly from matrices with ionic binding sites.[32,57]

Table 1 Arsenic separation and determination techniques

Reference	Matrix	Separation technique	Detection	Species determined[a]
21	DI water	HPLC(IC)	ICP-MS/Flame AAS	As(III), As(V), MMA, DMA, AsB
22	DI water	HPLC(IC)	Flame AAS	As(V), TMA, AsC
23	Urine, Marine org	HPLC(IC)	Flame AFS	As(V), MMA, DMA
24	DI water	HPLC(IC)	ICP-MS	As(III), As(V), MMA, DMA
25	DI water		HG-ETA	As(III), As(V)
26	River sediment	HPLC(IC)	HG-ETA	As(III), As(V), MMA
27		HPLC(IC)	GFAAS/thermospray MS	As(III), As(V), MMA, DMA, AsB, AsC
28, 29	DI water	HPLC (IC)	MO-HG-ICP-MS	As(III), As(V), MMA, DMA
30	Soil	HPLC(IC)	HG-FAAS	As(V)
31	Soil	HPLC(IC)	GFAAS	As(V), MMA, DMA
32	Soil	HPLC(IC)	DC-AES	As(III), As(V), DMA, MMA
33	Soil	HPLC(IC)	HG-AAS	As(III), As(V)
34	Urine	HPLC(IC)	UV-HG-AAS	As(III), As(V), MMA, DMA, TMAO, TMA, AsB, AsC
35	Marine organisms	HPLC(IC)	ICP-OES, HG-AAS	MMA, DMA, AsB, AsC
36	Flyash	HPLC(IC)	ICP-MS	As(III), As(V), MMA, DMA
37	Urine	HPLC(IC)	ICP-MS	As(V), DMA, arsenosugar metabolites
38	DI water	HPLC(IC)	HG-ICP-AES	As(III), As(V), MMA, DMA
39	DI water	GC-cryotrap	HG-ETA	MMA
40	Seaweed	GC	GFAAS, AAS	As(III), As(V), MMA, DMA
41	Solvent	GC	MIP-AES	TMAO
42	Marine organism tissues	HPLC	ICP-MS	DMA, TMA, AsB, As containing ribofuranosides

[a] As(III): inorganic arsenite, AsO_3^{2-}; As(V): inorganic arsenate AsO_4^{3-}; MMA: monomethyl arsonate, $CH_3AsO_3^{2-}$; DMA: dimethyl arsinate, $(CH_3)_2AsO_2^-$; TMAO: trimethyl arsenic oxide, $(CH_3)_3AsO$; TMA: tetramethyl arsonium, $(CH_3)_4As^+$; AsB: arsenobetaine, $(CH_3)_3As^+CH_2COO^-$; AsC: arsenocholine, $(CH_3)_3As^+CH_2CH_2OH$.

Table 2 Extraction techniques for organometallic compounds

Reference	Metallic species extracted	Matrix	Instrumentation and procedures	Extraction solvent
59	General organometallic	Fine sediment	Soaking, sonication	HCl(aq) then ethyl acetate
60	Organotin compounds	Fine sediment	Sonication	HCl then diethyl ether
61	Organotin compounds	Fine sediment	Sonication and centrifugation	1-butanol
62	Organotin compounds	Biological samples and sediment	Centrifugation, separatory funnel	Hexane or ethyl acetate or both after HCl, NaCl and H_2O
47, 51	Organoarsenicals	Marine animal tissue	Microwave assisted extraction	MeOH–H_2O
52	Organoarsenicals	Marine animal SRM	Accelerated solvent extraction	
56	Arsenicals	Soil	Sequential extraction	HCl, chloroform
57	Arsenicals	Soil, sediments	Microwave extraction	Phosphoric acid
53	Organoarsenicals	Shellfish	Sonication	MeOH and phosphate buffer
54	Organoarsenicals	Shellfish	Heat (85 °C, 3 h)	Chloroform–MeOH; then water
42	Organoarsenicals	Shellfish tissue	Sonication and centrifugation	MeOH–H_2O 1 : 1
28	Arsenicals	Soil	Sonication at 45 °C	Water
55	General organometallic compounds	Fine sediments	Sonication	HCl–MeOH and benzene
55	General organometallic compounds	Soils and sediments	Soxhlet apparatus	Acetic acid–toluene
63–65	Metalloporphyrins; diketonates, and dithiocarbamate and labile organometallic compound	Soils and sediments	SFE	Supercritical CO_2
66	Organotin compounds	Soil and sediment	SFE	Supercritical CO_2
67–69	Polyvalent inorganic metal ions	Soils and sediments of different organic contents	Centrifugation and agitation	Sequential inorganic solvents
70	Polyvalent inorganic metal ions	Soils	N/A	DTPA and EDTA
71	Polyvalent inorganic metal ions	Soil; soil surface	N/A	Ammonium acetate
72, 73	Organic and inorganic ions	Soil	Pretreatment with mercaptoethanol; Soxhlet apparatus	Sequential extractions with acids then increasingly polar organic acids
74	General organometallic	Reference materials	Review	Various strategies and considerations

Compared to pressurized solvent extraction, Soxhlet extraction or sonication, microwave assisted extraction (MAE) differs in that heating takes place internally rather than by convection. Perhaps more importantly, in a sealed vessel the system becomes pressurized when the sample–solvent system absorbs microwave radiation. In this enclosed system, higher temperatures can be achieved, having the effect of accelerating the desorption of the analyte into the extraction solvent. The higher pressures improve the solvent–matrix interaction and hence improve the extraction efficiency. The technique is quicker and more versatile than many traditional extraction techniques. For example, a methanol extraction and a nitric acid digestion can be performed in two vessels simultaneously within the same run. Alternatively, different extractions can be performed simultaneously which target different classes of analytes.

MAE has gained acceptance as a means of extracting organic pesticides and pollutants,[75,76] and has been used to remove organophosphorus compounds from lake sediment,[77] and organoarsenic compounds from marine life,[51] soils, and sediments[57] with some success.

A problem for method development in the area of trace speciation of organometallic compounds in environmental samples is that there are very few reference materials available to help with method validation. Currently there are no soil and sediment materials available for which the arsenic species content has been certified, though there are several such materials for which the total arsenic content is certified. One possible approach to the production of speciated reference materials is to determine the required species in existing SRM. We have shown[81] that NIST SRM 2704 (Buffalo River sediment) contains a measurable amount of DMA (extracted with the help of ultrasound by methanolic HCl, separated from other As-containing species by anion-exchange HPLC and measured by directly coupled ICP-MS). We have now extended this work by the development of an MAE procedure, and improved the detection capability of the chromatographic system by incorporating post-column hydride generation. Recovery and possible degradation were investigated for DMA added to SRM 2704 (which does not contain a detectable amount of DMA) and the procedure developed applied to the determination of MMA in three NIST SRM (2704, Buffalo River sediment; 1944, New York–New Jersey waterway sediment; and 2710, Montana soil with highly elevated traces).

Experimental

Instrumentation

Sonication extraction experiments were performed in 50 ml Erlenmeyer flasks sealed with Parafilm in a 1 l ultrasonic bath (E/MC RAI, model 450). A microwave extraction unit (Questron Q-max 4000, Mercerville, NJ) equipped with a laptop NEC286 personal computer was used for all MAE experiments. The unit's software was capable of monitoring pressure within a control vessel and controlling temperature by adjusting the microwave power output. All vessels were Teflon-lined high pressure vessels fitted with high pressure rupture disks. Pressure and temperature were calibrated using the manufacturer's protocols.

For HPLC, the column used was a Hamilton (Reno, NV) PRP-X100 anion-exchange column (150 mm × 4.1 mm id; 10 μm particle size). Mobile phase was delivered isocratically by a Waters 6000-A dual piston pump at a flow of 2.0 ml min^{-1}. The injection volume (manual Rheodyne six-port valve) was 20 μl.

The post-column derivatization manifold is shown in Fig. 1. A peristaltic pump (Ismatec) was used in the hydride generation manifold. All reaction manifold tubing and fittings were Teflon (0.5 mm id). The pulse dampers shown were made by connecting a 20 cm length of 0.5 mm plugged Teflon tubing to a tee-joint connection after the Tygon peristaltic pump tubing. The optimized reaction coil lengths and reagent flow rates are shown in Table 3. A glass bead gas–liquid separator was used prior to introduction into the mass spectrometer. Argon was supplied to the manifold and to the mass spectrometer through

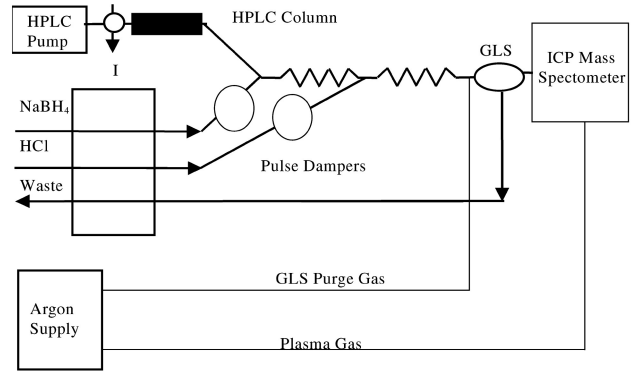

Fig. 1 Arsenic speciation manifold. Following separation, arsenic species are introduced to a hydride generation manifold. The arsine vapors generated in the reactor coil are then separated from the liquid in the gas–liquid separator (GLS) prior to introduction to the inductively coupled plasma mass spectrometer.

Table 3 Instrumentation parameters

A. Hydride generation	
Reaction coil length (0.5 mm dia)	1.5 m
Gas–liquid separator	glass bead
Hydrochloric acid concentration	25% (v/v)
HCl flow rate	7 ml min^{-1}
$NaBH_4$ concentration	1.5% (m/v)
$NaBH_4$ flow rate	4 ml min^{-1}
Waste flow rate	20 ml min^{-1}
Purge gas flow rate	0.900 l min^{-1}
B. HPLC	
Column	Hamilton PRP X-100, 10 μm anion-exchange column, 150 mm × 4.1 mm
Injection volume	20 μl
Flow rate	2.0 ml min^{-1}
Mobile phase	10 mM PO_4^{3-}, pH 6.0, isocratic
C. Mass spectrometer	
Coolant gas flow	15.0 l min^{-1}
Auxiliary gas flow	1.0 l min^{-1}
Purge gas flow	0.8–1.0 l min^{-1}
Mass/charge monitored	75
Collection frequency	8 Hz
Dwell time	0.125 s
Data collection mode	continuous
D. Microwave extraction program	
Maximum pressure	1150 kPa
Maximum temperature	160 °C
Power	1000 W
Start ramping time	0 min
Starting temperature	25 °C
End ramping time	6 min
Ending temperature	155 °C
Time held at final temperature	15 min

mass flow controllers. The dampers, inserted into the flow lines prior to the reaction confluence point, stabilized reagent deliveries improving the S/N, and preventing the plasma from being extinguished due to fluctuation in the hydrogen flow.

A PerkinElmer Sciex ELAN-5000 plasma source mass spectrometer, equipped with a laboratory-built Teflon hydride adapter, was used as the detector. The spectrometer settings are shown in Table 3. The spectrometer was controlled with an IBM 386DX computer and PerkinElmer ELAN software.

Chemicals and reagents

Stock solutions of 100 μg ml^{-1} arsenic as MMA, DMA, As(III) and As(V) were made and diluted to appropriate volume with deionized water (DI; E-pure, 18.0 MΩ cm^{-3}) before analysis. Standard solutions of 100 μg ml^{-1} arsenic as MMA (0.2506 g of $CH_3AsO_3Na_2$, ChemSource, 98%), arsenic as DMA (0.2915 g of $C_2H_6AsNaO_2.3H_2O$, Fluka, 98%), arsenic as inorganic As(III) (0.1737 g of NaAsO2, Mallincroft, analytical grade assumed 100%) and arsenic as As(V) (0.4164 g of $Na_2HAsO_4.7H2O$, Fisher, assumed 100%) were prepared in 1000 ml calibrated flasks. The HCl used was Fisher certified ACS plus grade, and the reductant used was NaBH4 (Alfa Aesar, granulated, 98% purity).

Extraction solvents for the MAE and sonication were prepared by mixing equal volumes of 10% (v/v) HCl and the pure solvent to give solutions containing 5% (v/v) HCl and 50% (v/v) organic modifier (on an volume/volume basis) in deionized water (E-pure, Millipore). The organic modifiers were Fisher HPLC-grade acetone, methanol, or isopropanol.

The chromatography mobile phase was 10 mM ammonium dihydrogen phosphate (BDH AnalaR Grade), adjusted to pH 6.0 with 0.1% NH_4OH (Fisher certified ACS grade).

Extraction

To evaluate the extraction efficiency, 0.5 g samples of SRM 2704 were weighed into either 25 ml Erlenmeyer flasks (for sonication), or PTFE lined vessels (for MAE). The samples were spiked with 200 μl of 100 μg ml^{-1} DMA stock solution. Deionized water (1.0 ml) was then added to the spiked sediments, and the contents were mixed with a polyethylene spatula to ensure homogeneity of the DMA. The samples were dried in an oven at 50 °C for 12 h and allowed to equilibrate for 6 d. After equilibration, approximately 10 ml of the extraction solvent was added to the vessels and flasks. For the sonication extraction, samples were sonicated for 2.0 h. For MAE, the extraction program shown in Table 3 was run.

Method validation and optimization

The HPLC conditions were selected on the basis of previous work. The Hamilton PRP-X100 column, a strong quaternary amine anion-exchanger, has been used in the separation of arsenic compounds by other investigators.[78–80] Kaise and co-workers have shown that a suitable mobile phase for the separation of arsenicals is phosphate at a pH of 6.0.[44–46]

In our preliminary investigations of arsenic species in NIST SRM 2704, the column eluent was directly aspirated.[81] Although this procedure was reproducible and gave good improved resolution it suffered from poor sensitivity and degraded the nickel sampler and skimmer cones of the mass spectrometer. On-line hydride generation of the column eluent eliminated these practical shortcomings. The manifold was optimized (also using the multicycle univariate method) so as to maximize the sensitivity of MMA and DMA peaks following chromatographic separation. The reaction coil length, borohydride concentration, and HCl concentration were varied until the maximum sensitivity for DMA and MMA were achieved. The reaction coil length was varied from 1–2 m, and the $NaBH_4$ concentration was varied from 0.5%–1.5% (w/v). The HCl concentration was varied from 5–25 % (v/v).

The extractability of MMA from SRM 2704, SRM 1944 and SRM 2710 was considered to be similar to the extractability of spiked DMA from SRM 2704. It is known that the humic matter, iron and other mineral contents all affect the degree of adsorption. The content of the SRM with respect to major and minor element composition is given in Table 4 from which it can be seen that the materials all have rather similar compositions. The natural deposition of MMA onto a sediment particle would be similar to the spiked deposition of the DMA. If the arsenic methylation was the result of microbial action, alkyl arsenic species are probably on the surfaces of sediment and soil particles, having been released in some way (excreted, expired, or secreted) by the microorganisms following transformation. It was also assumed, based on other work in our laboratory,[82] that conditions which would extract DMA would also extract MMA.

Multi-cycle univariate optimization of the mass spectrometer operating parameters (torch position, purge gas flow, and ion lens voltage settings) was performed daily as described by Greenfield, *et al*.[83] while a continuous stream of As(III) was introduced into the hydride generation manifold.

Calibration

Quantification of MMA in SRM 2704 was by external calibration and by internal standardization. The extraction efficiency used was assumed to be 100%, based on the calculated efficiency in the DMA recovery studies. The external calibration was performed by injecting a series of 5 standard solutions of MMA. These standard solutions were matrix matched with respect to acid and acetone content. A plot of peak area *vs*. concentration was constructed and the concentration of unknowns was calculated on the basis of rectilinear least squares regression.

As SRM 2704 did not contain a measurable amount of DMA, the concentration of MMA could be additionally quantified by a procedure in which DMA was used as an internal standard. A known volume of a known concentration of DMA solution was added to the extract following centrifugation. First, the response factors for DMA and MMA were calculated from the peak areas of the two compounds obtained from a 20 μl injection of a

Table 4 Elemental compositions (percent) of soils and sediment SRM

Element	SRM 2704	SRM 1944	SRM 2710
Aluminium	6.11	5.33	6.44
Silicon	29.08	31	28.97
Iron	4.11	3.53	3.38
Manganese	0.555	0.505	1.01
Magnesium	1.2	1.0[a]	0.853
Sodium	0.457	1.9	1.14
Potassium	2.00	1.6	2.11
Carbon	3.348	—[b]	3[a]

[a] Indicates material is not certified for this element. [b] —Value not available.

solution containing a known concentration of both species. These values were obtained before and after each analysis and the average ratio used in the calculation of the MMA concentration. The volume of the extractant was determined by subtraction of the mass of the centrifuge tube and dry sample from the total mass of the tube and contents after centrifugation, followed by division by the density of the extractant solution (0.90 mg ml^{-1}).

For SRM 2704 and 1944, four samples were taken, and for SRM 2710, three samples. Each sample was run twice and an average was taken.

Results and discussion

The parameters found to be the most important in maximizing the efficiency of the volatile hydride form of methylated arsenic species were the reaction coil length and the $NaBH_4$ concentration; the acid concentration had no significant effect on sensitivity and a value of 25% was used. The signals for the two methylated arsenic species showed almost identical variation with operating parameters. At borohydride concentrations above 1.5%, the hydrogen liberated from the $NaBH_4$–HCl reaction extinguished the plasma. Although at reaction coil lengths greater than 2 m improved hydride generation efficiency was found, the peak broadening was too great to resolve DMA from As(III), an especially prevalent problem as the column aged and lost efficiency. Since the decrease in sensitivity for 01.5 m reaction coil was quite modest, this was selected for the reaction coil. The optimized conditions produced signals from the methylated species which were about 15% of that obtained for the same concentration of As(III).

The peak area response was linear up to 500 ng ml^{-1} (R^2 = 0.9999) for both species. The 3*s* peak area detection limit was 1 ng ml^{-1} for MMA (approximately 10 ng g^{-1} in the solid) and 2 ng ml^{-1} for DMA (approximately 20 ng g^{-1} in the solid).

Extraction efficiency

The recoveries of DMA spiked into Buffalo River sediment are shown in Table 5 for various solvents. There was no evidence of DMA degradation for those solvent combinations which included HCl. These results together with the arsenic-specific chromatograms provide some insights into the extraction mechanisms. The presence of an organic modifier may aid in the solvent's ability to permeate (*i.e.* wet) the organic material in the soil, where the arsenic species could be adsorbed. Hydrochloric acid is able to protonate most solvents in which it resides as well as producing hydronium ions. These cationic species could help in solubilizing anionic arsenic species by ion-pairing. Simultaneously, chloride can serve as a strong electrolyte replacing anionic species retained at ion-exchange sites. The role of Cl^- in an ion-exchange process could partially account for the improvement in extraction efficiency compared to those of earlier investigations.[78] Additionally, there was little oxidative potential to any of the extraction solutions, so conversion of the arsenic species to inorganic As(V) was unlikely.

A significant amount of chromatographically unretained arsenic was observed in each of the extracts. It is noted that this unretained peak contains inorganic As(III), but also probably contains all other neutral or cationic arsenic species. Further separation of the co-eluting species was not attempted, and so the ability of different solvent systems to extract varying amounts of these arsenic species could not be evaluated. Varying the organic modifier hydrophobicity changes the speciation profile. It was noted that the more polar acetone–HCl and methanol–HCl systems removed comparatively more arsenate than did the isopropyl alcohol–HCl system, with less unretained arsenic compounds. The polarity of the modifier affects the solvent's ability to permeate the organic material of the soil, where the neutral arsenic species reside. A less polar solvent system would have poorer solvating ability for anions such as MMA and inorganic arsenate, and would be less efficient at removing them.

Near 100% recovery of spiked DMA was obtained with all three solvent systems. The aqueous acetone–HCl solvent system was chosen for the extraction of MMA from the other reference materials.

Determination of MMA

Table 6 shows the concentrations of MMA in the SRM, together with the percentage of the total arsenic that the MMA species contributes to the certified value. These concentrations were determined by external calibration, and assumed 100% extraction efficiency. The determination of DMA in SRM 2704 by internal standardization gave a value (0.40 ± 0.10 ppm) in agreement with the concentration found by external standardization. The internal standard method could not be used for SRM 2710 and 1944 because either there was a measurable amount of DMA in the extract (for SRM 1944), or because the unretained arsenic peak masked the retention position of the DMA (for SRM 2710).

The percentage of arsenic as MMA was remarkably similar for each of the materials coming from anaerobic environments (SRM 2704 and 1944). Although the MMA concentration was

Table 5 Extraction efficiencies (represented as DMA recovery in SRM 2704)

Extraction solvent	Method	Extraction efficiency (%)	Std Dev (%)
50% Acetone–50% methanol	Microwave	20	15 (n = 3)
	sonication	8	1.8 (n = 3)
Isopropanol	Microwave	15	8.2 (n = 3)
	sonication	9	3.6 (n = 3)
5% HCl–50% acetone (v/v)	Microwave	106	6.2 (n = 3)
	sonication	100	7.5 (n = 3)
5% HCl–50% methanol (v/v)	Microwave	112	17 (n = 3)
	sonication	124	16 (n = 3)
5% HCl–50% isopropanol(v/v)	Microwave	91	5.3 (n = 2)
	sonication	92	4.5 (n = 3)

Table 6 MMA concentrations (based on arsenic) in standard reference materials

Material	MMA concentration by ext. calibration (ppm) (±95% CI)	Certified total As conc. (ppm)	MMA as a percentage of total As
SRM 2704 (Buffalo River sediment)	0.30 ± 0.13 (n = 4)	23.4	1.2%
SRM 1944 (NJ/NY waterway sediment)	0.23 ± 0.12 (n = 4)	18.9	1.2%
SRM 2710 (Montana soil, highly elevated traces)	1.03 ± 0.15 (n = 3)	626	0.16%

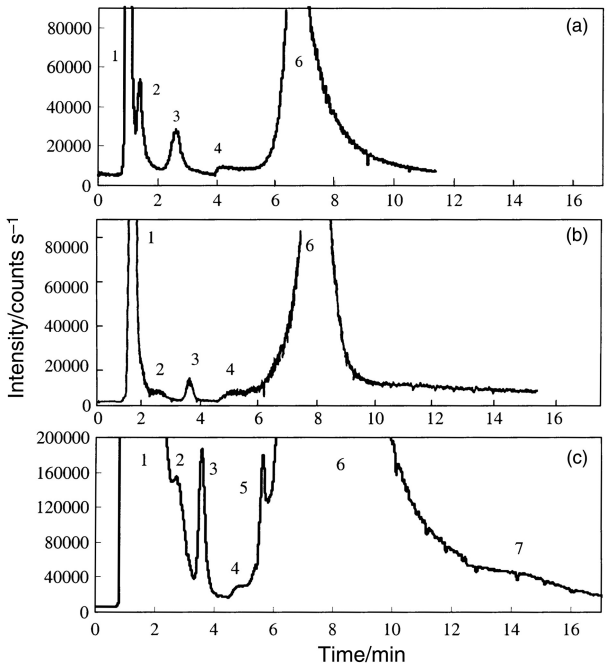

Fig. 2 Arsenic Speciation Profiles by HPLC-HG-ICP-MS. (a) SRM 2704 Buffalo River sediment; (b) SRM 1944 New York New Jersey waterway sediment; (c) SRM 2710 highly elevated Montana soil. 1. Unretained arsenic including AsO_3^{2-}; 2. DMA; 3. MMA; 4. Solvent impurity; 5. Unknown As species; 6. AsO_4^{3-}; 7. Unknown As species. Column: PRP-X100 anion-exchange column (10 μm particle size, 150 mm × 4.1 mm) Mobile phase: 10 mM phosphate buffer, pH 6.0. Flow rate 2.0 ml min^{-1}. Injection volume 20 μl.

highest in SRM 2710, the percentage of the total arsenic as MMA was about an order of magnitude lower than that for the reference materials taken from the anaerobic environments. While this could indicate that arsenic methylation is favored in an anaerobic medium, as has been found by other investigators who have studied biological arsenic methylation,[2–4] it is also possible that the mineral forms of arsenate in these media are more suitable for methylation.

Arsenic chromatographic profiles

The arsenic-specific chromatograms of the acetone–HCl solvent system microwave extracts of (a) Buffalo River sediment (SRM 2704), (b) New York-New Jersey waterway sediment (SRM 1944), and (c) highly elevated Montana Soil (SRM 2710), are shown in Fig. 2.

The identity of MMA in the microwave assisted and sonication sample extracts was verified by both matching retention times with those of the standards, and by adding an MMA spike to the sample and observing an increase in the size of the MMA peak. It is apparent from the arsenic profiles that there are differences in the retention times of different species between SRMs, most notably between SRM 2704 and the others. Retention time shifts like this have been observed by previous investigators, and could be due to the presence of other anions in the extract.[84] It was also observed that slight variations in mobile phase pH had a significant effect on the retention and separation of the arsenicals. Additionally, retention capacity and efficiency of the column changed over the course of the study.

The most stable form of terrestrial arsenic is inorganic As(v) as AsO_4^{3-}. Though it was not quantified in this investigation, this was the dominant form of extractable arsenic in all of the SRM investigated. There was evidence of a small amount of DMA in both the New York Harbor sediment (SRM 1944) and the Montana soil (SRM 2710), but the large peak for arsenic species eluting near the solvent front was not completely separated from the DMA peak. Future work to separate these unretained arsenic compounds (by reversed-phase or element-specific gas chromatography) is a possible extension to this study. There were a considerably greater number of separable arsenic species detected in the Montana soil extracts than in either of the sediment extracts. Of the three materials, only the soil was taken from an aerobic environment. Alternatively, these compounds could be related to an arsenic-bearing mineral.

The relatively small amounts of the methylated forms of arsenic suggest that for the materials investigated, there was no mechanism for the accumulation of these methylated species. Thus if microbial action was responsible for the conversion of inorganic arsenic to methylated arsenic either (a) MMA and DMA are more water soluble than arsenate or (b) MMA and DMA are intermediates in a pathway that produces volatile trimethylarsine or trimethylarsine oxide. Alternative biological pathways such as the production of arsenobetaine may be part of a closed cycle as it has been shown, for example, that in active anaerobic marine cultures, arsenobetaine degrades into a number of less substituted arsenic compounds including AsO_4^{3-}.[85] So far, little work has been done on the production and fate of methylated arsenicals in an aerobic environment.

Conclusions

Microwave-assisted extraction is a rapid, precise and quantitative procedure for extracting MMA from NIST soil and sediment reference materials. The dominant arsenic species in all extracts was inorganic AsO_4^{3-}. This species is the most

stable from a redox perspective, and is a likely candidate as a stable end-product of degradation. Quantifiable amounts of MMA were found in NIST SRM 2704, SRM 1944, and SRM 2710. The sediment reference materials, taken from environments that were predominantly anaerobic, had proportionally more arsenic as MMA than did the soil reference material, taken from an environment that was predominantly aerobic.

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References

1 F. Challenger, C. Higginbottom and L. Ellis, *J. Chem. Soc*., 1933, 95.
2 B. C. McBride and R. S. Wolfe, *Biochemistry*, 1971, **10**, 4312.
3 M. D. Baker, W. E. Innis, C. I. Mayfield, P. T. S. Wong and Y. K. Chau, *Environ. Technol. Lett*., 1983, **4**, 89.
4 W. R. Cullen and K. J. Reimer, *Chem. Rev*., 1989, **89**, 713.
5 H. Gurleyuk, V. Van Fleet-Stadler and T. G. Chasteen, *Appl. Organomet. Chem*., 1997, **11**, 471.
6 F. Challenger and H. E. North, *J. Chem. Soc*., 1934, 68.
7 H. E. Guard, A. B. Cobet and W. M. Coleman, *Science*, 1981, **213**, 770.
8 M. L. Bird and K. Challenger, *J. Chem. Soc*., 1939, 163.
9 U. Schmidt and F. Huber, *Nature*, (*London*), 1976, **259**, 157.
10 P. T. S. Wong, Y. K. Chau and P. K. Luxon, *Nature*, (*London*), 1975, **253**, 263.
11 M. Hiroki and Y. Yoshiwarn, *Soil Sci. Plant Nutr*., 1993, **39**, 243.
12 D. A. Bright, S. Brock, W. R. Cullen, G. M. Hewitt, J. Jafaar and K. J. Reimer, *Appl. Organomet. Chem*., 1994, **8**, 415.
13 E. H. Larsen, M. Hansen and W. Gossler, *Appl. Organomet. Chem*., 1998, **12**, 285.
14 M. Dannan, S. Dally and F. Conso, *Neurology*, 1984, **34**, 1524.
15 M. Vahter and E. Marafante, *Chem. Biol. Interact*., 1983, **47**, 29.
16 M. Tezuka, K. Hanoika, K. Yamanaka and S. Okada, *Biochem. Biophys. Res. Commun*., 1993, **191**, 1178.
17 J. R. Cannon, J. B. Saunders and R. F. Toia, *Sci. Total Environ*., 1985, **31**, 181.
18 G. B. Freeman, R. A. Schoof, M. V. Ruby, A. O. Davis, S. C. Liao, C. A. Lapin and P. D. Bergstrom, *Fundam. Appl. Toxicol*., 1995, **28**, 215.
19 R. Bornschein, *An Assessment of Residential Arsenic Exposure Among Children Living in the Vicinity of a Former Copper Smelter Plant*. Prepared for Atlantic Richfield Company, Denver, CO., University of Cincinnati, 1994.
20 S. Tamaki and J. R. Frankenberger, *Rev. Environ. Contam. Toxicol*., 1992, **124**, 79.
21 X. C. Le, W. R. Cullen and K. J. Reimer, *Talanta*, 1994, **41**, 495
22 E. H. Larsen and S. H. Hansen, *Mikrochim. Acta*, 1992, **109**, 47.
23 L. Ebdon, *Anal. Chim. Acta*, 1982, **136**, 191.
24 T. Shepard and J. Caruso, *Analyst*, 1992, **117**, 971.
25 L. Ebdon and S. Hill, *Anal. Proc*., 1986, **23**, 6.
26 K. Hanaoka, H. Yamamota, K. Kawashima, S. Tagawa and T. Kaise, *Appl. Organomet. Chem*., 1988, **2**, 371.
27 W. R. Cullen and M. Dodd, *Appl. Organomet. Chem*., 1989, **3**, 401.
28 D. Beauchemin, *J. Anal. At. Spectrom*., 1998, **13**, 1.
29 M. Moldovan, M. M. Gomez, M. A. Palacios and C. Camara, *Microchem. J*., 1998, **59**, 89.
30 S. H. Hansen, E. Larsen, G. Pritzl and C. Cornett, *J. Anal. At. Spectrom*., 1992, **7**, 629.
31 R. Indevia, N. Aharonson and E. A. Woolson, *J. Assoc. Off. Anal. Chem*., 1980, **63**, 742.
32 M. S. Mohan, R. A. Zingara, P. Micks and P. O. Clark, *Int. J. Environ. Anal. Chem*., 1982, **11**, 175.
33 B. A. Manning and D. A. Martens, *Environ. Sci. Technol*., 1997, **31**, 171.
34 D. L. Tsalev, M. Sperling and B. Welz, *Analyst*, 1998, **123**, 1703.
35 M. B. Amran, F. Lagarde and M. J. F. Leroy, *Mikrochim. Acta*, 1997, **127**, 195.
36 B. P. Jackson and W. P. Miller, *Environ. Sci. Technol*., 1999, **15**, 270.
37 X. C. Le, W. R. Cullen and K. J. Reimer, *Clin. Chem*., 1994, **40**, 617.
38 R. Rubio, A. P. Alberti and G. Rauret, *Mikrochim. Acta*, 1992, **109**, 39.
39 A. G. Howard and S. D. W. Comber, *Mikrochim. Acta*, 1992, **109**, 27.
40 L. Ebdon, S. Hill, A. P. Walton and R. W. Ward, *Analyst*, 1988, **113**, 1159.
41 K. B. Olsen, D. S. Sklarew and J. C. Evans, *Spectrochim. Acta, Part B*, 1985, **40**, 357.
42 Y. Shibata and M. Morita, *Appl. Organomet. Chem*., 1992, **6**, 343.
43 S. Maeda, A. K. Ohki, K. K. Miyahara, K. Naka and S. Higashi, *Appl. Organomet. Chem*., 1992, **6**, 415.
44 K. Hanaoka, S. Tagawa and T. Kaise, *Appl. Organomet. Chem*., 1991, **5**, 435.
45 K. Shiomi, Y. Horiguchi and T. Kaise, *Appl. Organomet. Chem*., 1988, **2**, 385.
46 T. Kaise and F. Shozo, *Appl. Organomet. Chem*., 1992, **6**, 155.
47 K. L. Ackley, C. B'Hymer, K. L. Sutton and J. A. Caruso, *J. Anal. At. Spectrom*., 1999, **14**, 845
48 K. Jin, T. Hayashi, Y. Shibata and M. Morita, *Appl. Organomet. Chem*., 1988, **2**, 365.
49 T. Tusuda, H. Nakanisi, S. Aoki and J. Takebayashi, *J. Chromatogr*., 1987, **387**, 361.
50 K. J. Lamble and S. J. Hill, *Anal. Chim. Acta*, 1996, **334**, 261.
51 T. Dagnac, A. Padro, R. Rubio and G. Rauret, *Anal. Chim. Acta*, 1998, **364**, 19.
52 J. W. McKiernan, J. T. Creed, C. A. Brockhoff, J. A. Caruso and R. M. Lorenzana, *J. Anal. At. Spectrom*., 1999, **14**, 607.
53 M. Stoeppler, M. Burow, K. May, S. Padberg and G. Kloster, *Mikrochim. Acta*, 1992, **109**, 107.
54 T. Kaise and F. Shozo, *Appl. Organomet. Chem*., 1992, **6**, 155.
55 R. M. Harrison and S. Rapsomanikis, *Environmental Analysis Using Chromatography Interfaced with Atomic Spectroscopy*, Ellis Horwood, Chichester, 1989.
56 J. Chappell, B. Chiswell and H. Olszowy, *Talanta*, 1995, **42**, 323.
57 P. Thomas, J. K. Finnie and J. G. Williams, *J. Anal. At. Spectrom*., 1997, **12**, 1367.
58 M. Burguera and J. L. Burguera, *Talanta*, 1997, **44**, 1581.
59 D. Velez , N. Ybanez and R. Montoro, *J. Anal. At. Spectrom*., 1996, **11**, 271.
60 M. D. Mueller, *Fresenius' Z. Anal. Chem*., 1984, **317**, 32.
61 K. S. Epler, T. C. O'Haver, G. C. Turk and W. A. MacCrehan, *Anal. Chem*., 1988, **60**, 2062.
62 T. Tsuda, H. Nakanishi, T. Morita and J. Takebayashi, *Assoc. Off. Anal. Chem*., 1986, **69**, 981.
63 M. Lee and C. E. Makides, *Analytical Supercritical Fluid Chromatography and Extraction*, Chromatography Conferences, Provo, UT, 1990.
64 C. Berger and M. Perrut, *J. Chromatogr*., 1990, **28**, 468.
65 B. A. Charpentier and M. R. Senenats, *Supercritical Fluid Extraction and Chromatography*, ACS Symposium Series, Washington, 1988.
66 Y. Liu, V. Lopez-Avila and M. Alcaraz, *J. High Resolut. Chromatogr*., 1993, **16**, 106.
67 A. Tessier, P. G. C. Campbell and M. Bisson, *Anal. Chem*., 1979, **51**, 844.
68 R. F. Sanzalone and T. T. Chao, *Soil Sci. Soc. Am. J*., 1989, **53**, 385.
69 S. Xiao-Quan and B. Chen, *Anal. Chem*., 1993, **65**, 802.
70 I. Novozamsky, T. M. Lexmond and V. J. G. Houba, *Int. J. Environ. Anal. Chem*., 1993, **51**, 47.
71 P. Del Castilho and I. Rix, *Int. J. Environ. Anal. Chem*., 1993, **51**, 59.
72 D. E. Wells, *Mikrochim. Acta*, 1992, **109**, 13.
73 L. Orsini and A. Bermond, *Int. J. Environ. Anal. Chem*., 1993, **51**, 97.
74 P. E. Gardiner, *Fresenius' J. Anal. Chem*., 1993, **345**, 287.
75 V. Lopez-Avila, R. Young and W. Beckert, *Anal. Chem*., 1994, **66**, 1097.

76 F. I. Onuska and K. A. Terry, *Chromatographia*, 1993, **36**, 191.
77 H. De Geus, B. N. Zegers, H. Lingeman and U. A. Th. Brinkman, *J. Environ. Anal. Chem.*, 1994, **56**, 119.
78 G. Rauret, R. Rubio and A. Padro, *Fresenius' J. Anal. Chem.*, 1991, **340**, 157.
79 E. H. Larsen and S. H. Hansen, *Mikrochim. Acta*, 1992, **109**, 47.
80 D. Velez, N. Ybanez and R. Montoro, *J. Anal. At. Spectrom.*, 1996, **11**, 271.
81 P. M. Yehl and J. F. Tyson, *Anal. Commun.*, 1997, **34**, 49.
82 H. Gurleyuk, PhD Thesis, University of Massachusetts, 2001.
83 S. Greenfield, M. S. Salman, M. Thomsen and J. F. Tyson, *J. Anal. At. Spectrom.*, 1989, **4**, 55.
84 A. M. Debettencourt, M. H. Florencio, M. F. N. Duarte, M. L. R. Gores and L. F. C. V. Boas, *Appl. Organomet. Chem.*, 1994, **8**, 43.
85 S. Maeda, K. Kusadome, H. Arima, A. Ohki and K. Naka, *Appl. Organomet. Chem.*, 1992, **6**, 407.