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Determination of four arsenic species in soil by sequential extraction and high performance liquid chromatography with post-column hydride generation and inductively coupled plasma optical emission spectrometry detection‡

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Several procedures, involving various solvents and ultrasound, were evaluated for the extraction of four arsenic species, arsenite (As(III)), arsenate (As(V)), monomethylarsonate (MMA) and dimethylarsinate (DMA), from a silt loam soil to which species had been added at a concentration of 20 mg kg^{-1}. The best extraction was by a two-stage procedure: shaking for 24 h in the presence of 0.1 mol l^{-1} phosphoric acid followed by shaking for 24 h in 1.0 mol l^{-1} sodium hydroxide solution. The arsenic species in the extracts were separated by high performance ion-exchange liquid chromatography, derivatized to hydrides by reaction with tetrahydroborate(III) in a multi-mode sample introduction system (MSIS) and quantified by ICP-OES. Detection limits in solution ranged from 0.4 (As(III) and DMA) to 1 (MMA and As(V)) μg l^{-1}, corresponding to 10 and 25 μg kg^{-1} in a 0.2 g soil sample and 5 ml of extractant. The most significant change over time was that As(III) was converted to As(V). When each species was added individually, arsenic was 100% recovered over a period of several months. When all four species were added together, the recovery was 89%. As the precipitation of humic acids was slow, the sodium hydroxide extract could be acidified and analyzed without loss of analyte species.

Introduction

Soil, a complex heterogeneous mixture of minerals, organic solids, aqueous and gaseous components, is the medium in which not only plants grow, but also dead organisms are degraded and recycled. The mineral fraction contains weathered rock fragments consisting of phyllosilicates (silicate minerals are the largest and most important mineral class), clay minerals, oxides (mainly of aluminium, iron, and manganese) and various carbonates. The organic matter is made up of living organisms, dead plant material and colloidal humus formed by the action of micro-organisms on plant litter. Water and air usually fill the pores created when the solid components cluster together.[1]

About 99% of the arsenic (the 20th most abundant element in the earth's crust[2]) in the environment is associated with rocks and minerals; arsenopyrite (FeAsS) being the most abundant arsenic-containing mineral.[3] Other important minerals that can contain arsenic include arsenolite (As_2O_3), olivenite (Cu_2OHAsO_4), cobaltite (CoAsS), and proustite (Ag_3AsS_3).[3] The concentrations of arsenic in the earth's crust, shale, sedimentary rocks, and igneous rocks are 1.8, 13, 1.7–400, and 1.3–3.0 μg g^{-1}, respectively.[4] The background concentration of arsenic in soils, which depends on the rock type, ranges from 1–40 mg kg^{-1} with most soils being in the lower half of this range.[5] The predominant forms of arsenic in soil are the inorganic forms of As(III) and As(V),[6,7] though methylated arsenic compounds, such as MMA and DMA, can be formed by microorganisms under favorable conditions.[8–11] Arsenic compounds that function as herbicides, fungicides or insecticides (such as cacodylic acid and lead arsenate) may be found in soils as the result of topical applications to plants, the leaching of arsenic compounds from timber pressure-treated with chromated copper arsenate (CCA) or irrigation with arsenic-contaminated ground water. Soils may also contain arsenic compounds that come from mine wastes, industrial wastes, chemical warfare agents or the application of manure that contains veterinary drug residues. Arsenic compounds from the combustion of fossil fuels can be deposited from the atmosphere.[12] Arsenic concentrations in such contaminated soils can range from a few hundred to several thousand mg kg^{-1}.[7]

As the bioavailability and toxicity of arsenic compounds vary dramatically, the assessment of arsenic transport, environmental impact and human health risk should be based on measurement of arsenic speciation as well as total arsenic concentration.[13] In addition, studies of the transformation of arsenic compounds need to be supported by reliable measurements of arsenic species.

There is a sustained, increasing interest in the chemical measurement aspects of arsenic speciation as evidenced by publication rate. The vast majority of the published papers describe the coupling of HPLC with ICP-OES or ICP-MS. Since the first such publication in 1984,[14] almost 600 such publications have appeared; since 2002, over 50 papers per year have been published. Developments can be followed in the regular annual review literature,[15] and have been recently reviewed.[16–19] About

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‡ Electronic supplementary information (ESI) available: Effect of (a) $NaBH_4$ concentration, (b) $NaBH_4$ flow rate, (c) hydrochloric acid flow rate, (d) nebulizer flow rate, (e) plasma viewing distance, and (f) RF power (Fig. S1). The effect of sonication time on the oxidation of 1.0 mg l^{-1} As(III) prepared in (a) deionized water, (b) 0.01 mol l^{-1} NaOH and (c) 0.01 mol l^{-1} H_3PO_4 (Fig. S2). See DOI: 10.1039/b820300h

80% of these publications report on the development or use of a method in which ICP-MS is used as the detector, from which it is clear that ICP-MS has one or two drawbacks as an element-specific detector for HPLC: the instrument is not able to handle a wide range of mobile-phase compositions and some efforts have to be made to prevent the interference from chlorine, which produces an isobaric overlap, in quadrupole instruments, at *m*/*z* 75 due to the formation of $^{40}Ar^{35}Cl^{+}$ in the spectrometer. Detection by the more robust ICP-OES does not suffer from these problems, but the detection capability is inherently poorer than that of ICP-MS. For many analyses, this may not be the limiting factor, as the arsenic species are present in relatively high concentrations. There are smaller numbers of papers describing the use of AFS detection, almost all of which involve hydride generation, as the hydrogen diffusion flame atomizers typically used cannot tolerate the introduction of solution aerosol.[20–22]

There is also considerable interest in the determination of arsenic and arsenic species in soil.[23] Procedures may be divided into those based on selective, sequential extraction that are designed to estimate how much arsenic may be available for uptake by plants and those designed to extract all species so that they can be separated and quantified. While these latter procedures are, in principle, more useful there are considerable difficulties with ensuring that (a) all of the relevant species are extracted, and (b) there is no interconversion. Many studies of arsenic in the environment are concerned with following the fate of compounds that have been added from external sources, for which it is reasonable to assume that the species are bound to the surfaces of soil particles (either mineral or organic). Thus the sample pretreatment needed is to transfer (without change, other than protonation or deprotonation) the surface-bound species into solution. Experiments with model systems may be confounded by the gradual conversion of arsenic species into refractory minerals.[24] Various combinations of nitric acid, hydrogen peroxide and hydrofluoric acid are suitable for total arsenic determinations but convert all species to As(v). Milder procedures involve leaching with dilute mineral acids[25–28] (phosphoric, hydrochloric, perchloric or nitric), water, or solutions of ammonium acetate, acetic acid, ammonium chloride, citric acid, ammonium oxalate, sodium carbonate, sodium bicarbonate or sodium hydroxide.[25,27–30] Mixtures of phosphate with either ethylenediaminetetraacetic acid, hydroxylamine hydrochloride, or sodium diethyldithiocarbamate have been evaluated.[30]

Karthikeyan and Hirata[31] reviewed procedures for arsenic speciation in soil samples. Low recoveries (20–70%) were observed and, although the most effective extractant would appear to be phosphoric acid with low power microwave heating, As(III) is partially oxidized to As(V).[31] Procedures have involved sonication,[27,32,33] microwave assisted extraction,[34–36] or sequential extraction.[35,37] To date, there does not appear to be a satisfactory procedure for the extraction of arsenic species from soils as part of an analytical method. The situation may be different if the goal is washing for remediation purposes.[12]

We have evaluated several procedures, including the use of mechanical shaking, sonication and sequential extraction with a variety of solvents for the extraction of four species, As(III), As(V), DMA, and MMA, from soils for subsequent determination by HPLC-ICP-AES. We have devised a two-stage sequential procedure to extract these species quantitatively from spiked soil samples over a period of several months with minimal conversion during the extraction. We think this procedure is significantly better than any previously reported for the extraction of these species from soils. We have also developed an ion-exchange HPLC separation procedure for these four compounds in the extracts, that gives separations with a resolution of 1.4 or better in under 10 min, in which the multimode sample introduction system (MSIS),[38] in HG mode, functioned as the interface between the chromatographic separation and the spectrometer. To our knowledge, this is the first time that the use of the MSIS as a chromatographic interface has been reported.

Experimental

Instrumentation

The HPLC detector was an inductively coupled plasma optical emission spectrometer Optima 4300 DV (PerkinElmer Instruments, Shelton, CT, USA). Analytes were introduced into the spectrometer as hydrides with the multimode sample introduction system (MSIS) (PerkinElmer Instruments, Shelton, CT, USA). The chromatographic system consisted of a liquid chromatography pump (Finnigan SpectraSYSTEM P2000 Binary Gradient Pump) and an autosampler with a built-in injector valve (Finnigan SpectraSYSTEM AS3000 Autosampler), both were supplied by the ThermoElectron Corporation, Waltham, MA, USA. The column used was an Alltech Anion HC column (4.6 × 100 mm) (Alltech Associates, Inc., Deerfield, IL, USA) packed with a polystyrene divinylbenzene-based anion-exchanger, having a quaternary amine functional group, capable of operating over a pH range of 2–12, of particle size 12 μm, and capacity 0.3 meq g^{-1}.

The outlet of the column was connected to a T-junction at which concentrated hydrochloric acid, delivered by a peristaltic pump (Ismatec SA-MS-Reglo peristaltic pumps, Cole Parmer), was merged. Connecting tubing was made of polyether ether ketone (PEEK) or Teflon. The HPLC-ICP-OES system is shown schematically in Fig. 1. All mobile phases, standards and samples were filtered through 0.45 μm polyethersulfone membrane filters (Whatman Inc, USA) and degassed in an ultrasound bath (Fisher scientific, USA) prior to analysis.

The pH during the pH adjustment of the solutions was monitored with a Fisher Scientific model 915 meter. An ultrasonic probe (Sonics and Materials Inc. Danbury, CT, USA), Lab Quake shaker rotisserie (Barnstead-Thermolyne, USA), conventional microwave oven (Model MU3050W from Samsung) and a MDS 4100 microwave oven (CEM Corporation, USA) with PTFE vessels were used during sample preparation. Data from the spectrometer were collected with WinLab32 software (PerkinElmer Instruments, Shelton, CT, USA), processed with OriginPro 7.5 (OriginLab Corporation, Northampton, MA) and plotted with Microsoft Excel software.

Reagents and samples

All solutions were prepared in 18 MΩ cm deionized water from a Barnstead E-pure system (Barnstead, USA). Phosphoric acid (EM Science, Germany) and ammonium hydroxide (EMD Chemicals, USA) were used for pH adjustment. Phosphate buffer was prepared from ammonium dihydrogen phosphate (AnalaR, BDH Chemicals, UK), and phosphoric acid (EM

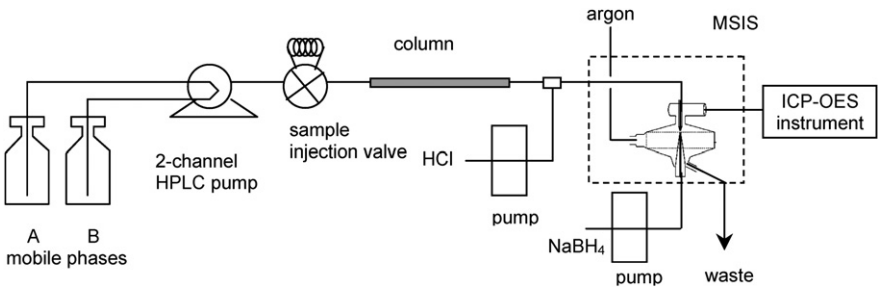

Fig. 1 Schematic diagram of the HPLC-HG-ICP-OES system. The sample loop size was 100 μl, MSIS is the multimode sample introduction system. The column was an Alltech Anion HC column.

Science, Germany). Solid reagents, sodium hydroxide, ammonium carbonate, ammonium bicarbonate, and ammonium acetate were obtained from Mallinckrodt, USA. Solutions of sodium tetrahydroborate (Alfa-Aesar, Ward Hill, MA) were freshly prepared daily by dissolving the appropriate amount of $NaBH_4$ in 0.1% (w/v) sodium hydroxide. The daily working standards for arsenic species were made from stock solutions (1000 mg l^{-1}) prepared from sodium arsenite ($NaAsO_2$) (Aldrich, USA), sodium arsenate ($Na_3AsO_4 \cdot 7H_2O$) (Fisher Scientific, USA), disodium methyl arsenate [$(CH_3)AsO_3Na_2 \cdot 6H_2O$] (ChemService, USA) and cacodylic acid [$(CH_3)_2AsO(OH)$] (Aldrich, USA) by dissolving the accurately weighed solid material in deionized water. These stock solutions were kept at 4 °C in the dark.

The soil was obtained from the Department of Plant, Soil and Insect Science, University of Massachusetts, Amherst, USA. The soil was a silt loam with 35.5% sand, 59.8% silt and 4.7% clay. It had an average pH of 6.8 and contained 1.3% organic matter. The full characterization is provided in the ESI‡.

Optimizing the MSIS hydride generation interface

All HG-ICP-OES parameters including, plasma viewing distance, RF power, nebulizer (argon gas) flow rate, sodium tetrahydroborate concentration and flow rate, on-line hydrochloric acid flow rate used for HG were optimized by a single-cycle alternating variable search method for a 0.1 mg l^{-1} As(III) standard solution at a flow rate of 1 ml min^{-1}. Starting conditions were based on the preliminary studies of the MSIS hydride generation method, and the figure of merit to be maximized was net signal.[38]

Separation of arsenic species with Alltech Anion HC column

The column was regenerated before use and as necessary according to the manufacturer's recommendation by passing 100 ml of a solution containing 50 mmol l^{-1} disodium EDTA adjusted to pH 10 with NaOH at flow rate 1 ml min^{-1}, rinsing with 100 ml deionized water, followed by 50 ml of 50 mmol l^{-1} sulfuric acid in 10% methanol at 1 ml min^{-1}, rinsing with 100 ml deionized water and then finally with mobile phase A (10 mmol l^{-1} ammonium dihydrogen phosphate at pH 5.8) for 15 min.

The chromatographic conditions previously developed[28] for the PRP-X100 column were selected. The mobile phase was at flow rate 1.0 ml min^{-1} with isocratic elution. Different concentrations of either individual species or mixtures of As(III), As(V), DMA, and MMA standards in deionized water were utilized for the development of separation conditions that gave good baseline separation. Concentrations of ammonium dihydrogen phosphate from 2 to 100 mmol l^{-1} were evaluated as a mobile phase with isocratic and gradient separation modes. Sodium hydroxide over the concentration range from 2 to 100 mmol l^{-1} was also evaluated as a mobile phase in isocratic mode.

Several gradient elution modes involving 10 mmol l^{-1} ammonium dihydrogen phosphate solution and water were evaluated, and the program that had the shortest analysis time with good resolution was chosen as optimal.

Under optimum conditions, shown in Table 1, calibration data for arsenic species containing 0.0, 0.1, 0.5, and 1.0 mg l^{-1} of a mixture of As(III), DMA, MMA, and As(V) standards were obtained. The detection limits of each species were calculated as the concentrations that give signals equal to three times the standard deviations of the blanks. Quantification was based on peak area measurement. Column efficiency (number of theoretical plates based on the peak that eluted between 8 and 10 min), detection limits and resolutions obtained were compared with those for other arsenic speciation techniques developed previously.

Table 1 Optimum parameters used for HPLC-HG-ICP-OES

HPLC-MSIS-HG-ICP-OES	
RF power/W	1400
Plasma view distance	−4
Nebulizer Flow rate/l min^{-1}	0.55
$NaBH_4$ concentration (w/v %)	1.5
$NaBH_4$ flow rate/ml min^{-1}	1.5
HCl flow rate/ml min^{-1}	0.1
Arsenic wavelength/nm	228.812
HPLC	All HPLC systems were operated at ambient temperature
Sample loop size/μl	100
Anion-exchange HPLC	
Column	Alltech Anion HC
Mobile phase	A: 10 mmol l^{-1} ammonium dihydrogen phosphate (pH 5.8), B: deionized water
Gradient program	
Time/min	0 (0% A–100% B) flow rate 1 ml min^{-1} 3 (0% A–100% B) flow rate 1 ml min^{-1} 8 (100% A–0% B) flow rate 1 ml min^{-1} 8.2 (100% A–0% B) flow rate 2 ml min^{-1} 10 (100% A–0% B) flow rate 2 ml min^{-1} 11 (0% A–100% B) flow rate 1 ml min^{-1}

Preparation of arsenic-spiked soil

Soil was sterilized by placing 500 g in a plastic container containing 500 ml deionized water in a conventional microwave oven and heated at full power for 10 min. The soil was transferred into an aluminium baking dish and dried in an oven for one week at 70 °C. The soil was ground, passed through a 250 μm sieve and 50 g was weighed into five 400 ml beakers to which 200 ml of deionized water was added. To four of the beakers was added, with continuous stirring, 1.0 ml of a solution containing 1000 mg l^{-1} (as As) of sodium arsenite, sodium arsenate, disodium methyl arsenate and cacodylic acid to produce soils containing 20 mg kg^{-1} as arsenic. To the fifth was added 1 ml each solution to produce a soil containing 80 mg kg^{-1} arsenic in total. The soils were dried at 70 °C for one week (Garcia-Manyes *et al.* reported[26] that soils heated at 100 °C did not lose arsenic). The dried soils were kept at room temperature in sealed 100 ml polypropylene containers in the dark until needed.

Extraction of arsenic species

The most promising of the previously reported extractants,[25,27–30] solutions of ammonium acetate, ammonium bicarbonate, ammonium carbonate, phosphoric acid, and sodium hydroxide were prepared at different concentrations (0.10, 0.50 and 1 mol l^{-1}) and evaluated, together with water, for the extraction of arsenic species. Accurately weighed 0.2 g arsenic-spiked soil samples were transferred to 15 ml centrifuge tubes to which was added 5 ml of the extractant solution followed by shaking for 24 h, centrifugation at 7000 rpm for 10 min, filtration from a 5 ml syringe through a 0.45 μm filter and determination of total arsenic by ICP-OES. Calibration curves were generated from seven standards (0.0, 0.05, 0.1, 0.3, 0.7, 1.0, and 2.0 mg l^{-1}) prepared for each species. Standards were matrix matched with respect to solvent composition. The two solvents that gave the highest extractions, phosphoric acid and sodium hydroxide were chosen for further investigation.

Sequential extraction method

Preliminary experiments were performed by shaking or with the help of an ultrasonic probe for different concentrations of phosphoric acid and sodium hydroxide. The soil spiked with As(III) was used, as the goal was to evaluate the stability of As(III) as well as the extraction efficiencies. The results of sequential extraction by 0.10 mol l^{-1} H_3PO_4 and 0.1 mol l^{-1} NaOH with sonication, sonication in an ice bath, or shaking were compared and the methods that gave the highest extraction efficiency with minimum As(III) oxidation were evaluated for all soil samples.

For sequential extraction, accurately weighed 0.2 g arsenic-spiked soil samples were transferred to 15 ml centrifuge tubes to which was added 5 ml of the 0.10 mol l^{-1} phosphoric acid solution followed by shaking for 24 h, centrifugation at 7000 rpm for 10 min, filtration from a 5 ml syringe through a 0.45 μm filter and the filtrate injected into an HPLC column for speciation analysis. Calibration curves were generated at four different concentrations (0.00, 0.10, 0.50 and 1.0 mg l^{-1}) prepared in 0.10 mol l^{-1} phosphoric acid. To the remaining soil, 5 ml of 0.10 mol l^{-1} sodium hydroxide was added and the tube shaken for 24 h then centrifuged for 10 min at 7000 rpm. The solution was filtered through a 0.45 μm filter, and the filtrate adjusted to pH 2.5 with 10% phosphoric acid. The resulting solution was injected into an HPLC column for arsenic speciation analysis. As a dark brown precipitate formed slowly following adjustment of the pH of the sodium hydroxide extracts to 2.5, this solution was analyzed immediately after the pH adjustments. Calibration curves of each species were generated for four different concentrations (0.00, 0.10, 0.50 and 1.0 mg l^{-1}) prepared in 0.10 mol l^{-1} sodium hydroxide subsequently adjusted to pH 2.5 with phosphoric acid. Three replicate extractions were made. The total arsenic concentration in the soil based on the sum of the arsenic present in the extracts was compared with the known concentration

Stability of arsenic species during the extraction

As there is evidence that ultrasound speeds up the extraction considerably compared with methods involving mechanical shaking,[27,33] the effect of sonication on the oxidation of As(III) was evaluated for 1.0 mg l^{-1} As(III) standards prepared in water, 10 mmol l^{-1} phosphoric acid, and 10 mmol l^{-1} sodium hydroxide. The probe was introduced into the solution and sonication was applied for between 1–15 min at 70% power. The peak height signals for As(III) and As(V), obtained by HPLC-HG-ICP-OES, were recorded.

Solutions containing 1.0 mg l^{-1} As(III), DMA, MMA, As(V), or a mixture of each species prepared in 0.10 mol l^{-1} phosphoric acid or 0.1 mol l^{-1} sodium hydroxide experiments were shaken, for 24 h. Species were determined for all solutions by HPLC-HG-ICP-OES.

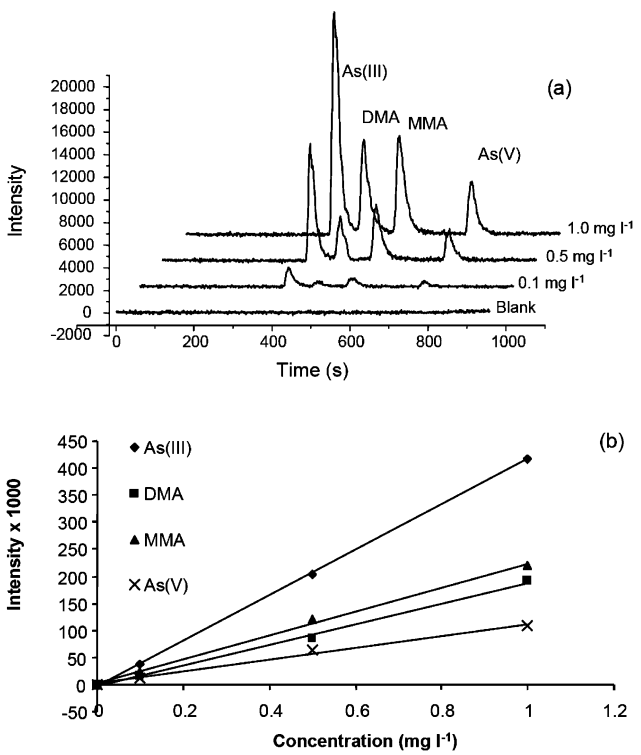

Fig. 2 (a) Chromatograms of standard solutions, (b) peak area calibration plots for each species.

Table 2 Comparison of detection limits and number of theoretical plates obtained by the method developed and those of previously published arsenic speciation techniques

Techniques	Reference	Column	N^a	LOD/μg l^{-1}			
				As(III)	DMA	MMA	As(V)
HPLC-HG-AAS	39	Hamilton PRP-X 100 (250 × 4.1 mm, 10 μm)	4138	2.4	2.3	2.4	2.6
HPLC-HG-AFS	40	Hamilton PRP-X 100 (125 × 4 mm, 5 μm)	NF[b]	0.9	1.4	0.8	1.0
HPLC-ICP-MS	27	Hamilton PRP-X 100 (150 × 4.1 mm,10 μm)	1418	0.1	0.12	0.13	0.15
HPLC-ICP-MS	41	Dionex Ion Pac AS7 (250 × 4 mm, 10 μm)	2317	0.19	0.16	0.29	0.52
HPLC-HG-ICP-OES	This work	Alltech Anion HC (100 × 4.6mm, 12 μm)	4010	0.36	0.41	0.9	1.1

[a] N: Number of theoretical plates. [b] NF: No figure.

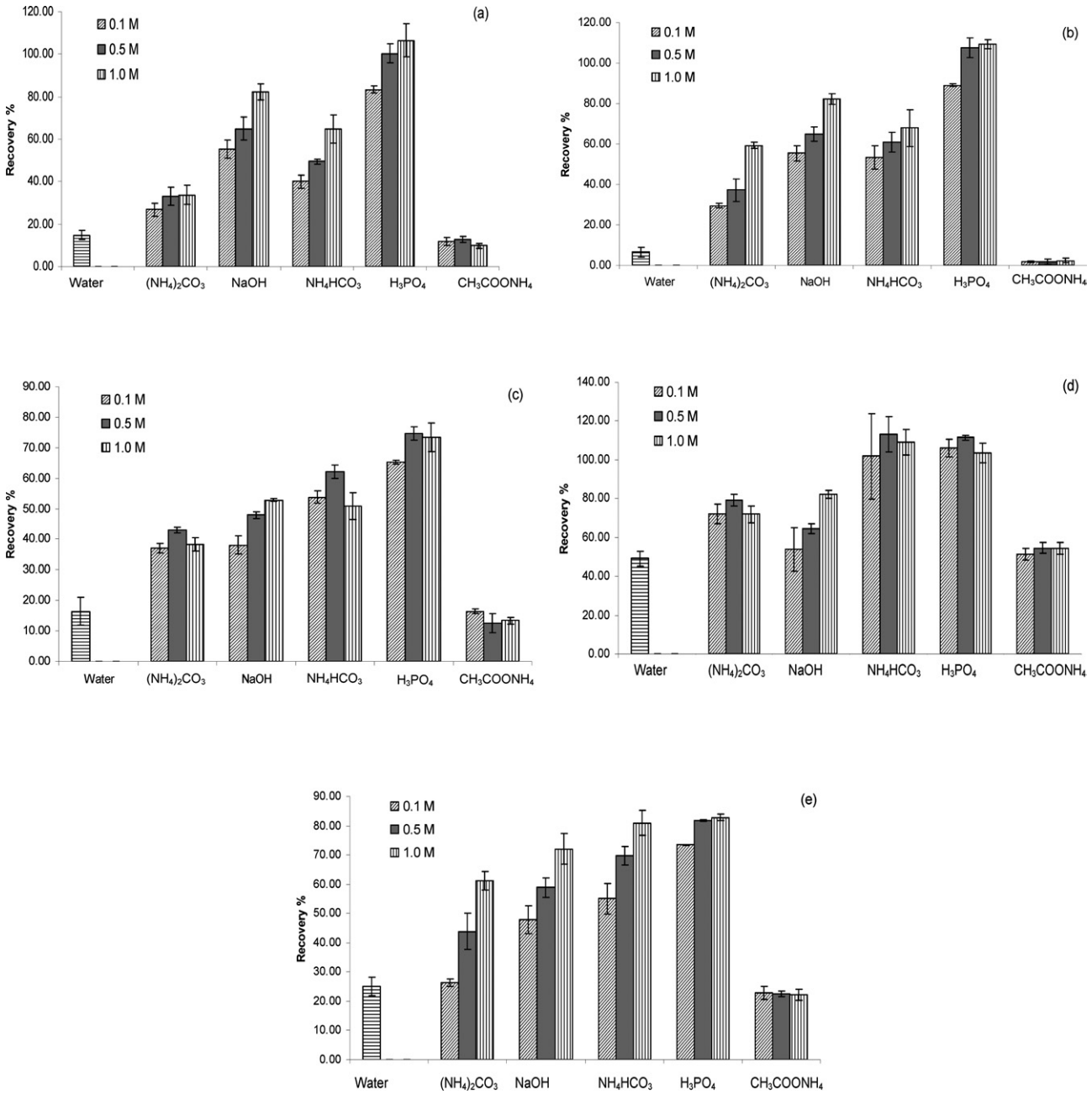

Fig. 3 Efficiencies of different solvents for extracting arsenic species from soil containing 20 mg kg^{-1} (a) As(III), (b) As(V), (c) MMA, (d) DMA and (e) a mixture of all four species. Error bars ± one standard deviation ($n = 3$).

Results and discussion

Optimizing the MSIS hydride generation interface

The optimum values are given in Table 1. Further details of the effects of the individual parameters are provided in the supplementary material.

Separation of arsenic species with Alltech Anion HC column

The optimum conditions chosen are given in Table 1 and the chromatograms and calibration plots are shown in Fig. 2. The variation in sensitivity, due to the variation in hydride generation efficiency, can be clearly seen. The chromatographic figures of merit and comparisons with other published HPLC procedures are shown in Table 2. It can be seen that although the MSIS device does cause some peak broadening, the efficiency of the separation is better than several previously reported systems with conventional nebulizer introduction. Resolutions between the peaks for As(III) and DMA, DMA and MMA, and MMA and As(V) were 1.4, 2.9, and 3.6, respectively.

Extraction of arsenic species

The extraction efficiencies of each solvent are shown in Fig. 3, from which it can be seen that phosphoric acid solution was the most effective extractant, giving almost 100% removal for all arsenic species, except MMA, when a concentration of 1.0 mol l^{-1} was used. Even 0.10 mol l^{-1}, H_3PO_4 removed more than 80% arsenic. The dissociation constants for phosphoric acid (pK_a 2.12, 7.2 and 12.4) are similar to those of arsenic acid (pK_a 2.2, 6.97 and 11.53). Therefore, similar charged species of arsenate and phosphate will be competing for the sorption sites on the soil components. Melamed *et al.*[42] found that As(V) mobility was greatly enhanced by treatment with increasing amounts of phosphate due to competitive oxyanion adsorption. Similarly, it has been found that phosphate substantially suppresses As(V) adsorption by the soil.[43,44] Wenzel *et al.*[45] suggested that because of the smaller size of phosphate, compared to that of arsenate, and its higher charge density, the phosphate will bind more strongly than arsenate and therefore, will replace the anionic forms of arsenic species in the soil. Sodium hydroxide solutions showed the second highest extraction efficiency for inorganic arsenic species; about the same as that of ammonium bicarbonate solutions for the extraction of MMA and poorer efficiency for the extraction of DMA than that of sodium bicarbonate. Since phosphoric acid and sodium hydroxide solutions exhibited the highest extraction efficiencies averaged over all arsenic species, they were selected for further studies and for the sequential extraction method.

Arsenic(III) in soil by sequential extraction

The effects of solvent type, concentration, and sequence on the extraction of As(III) and oxidation to As(V) from soil containing only As(III) are shown in Table 3. Even when solution was cooled in an ice bath, As(V) was detected. Sequential extraction by 0.10 mol l^{-1} H_3PO_4 and 0.10 mol l^{-1} NaOH with 24 h shaking gave the highest recovery for total arsenic. Therefore, sequential extraction by 0.10 mol l^{-1} H_3PO_4 and 0.10 mol l^{-1} NaOH with 24 h shaking was further evaluated for all spiked-soil samples.

Stability of arsenic species during extraction

The effect of sonication on the stability of As(III). The chromatograms, provided in the ESI‡, clearly show that the As(V) signal increased with time for all matrices indicating that sonication caused oxidation of As(III). After 15 min sonication in 10 mmol l^{-1} sodium hydroxide solution over half the As(III) had been oxidized to As(V). It was noted that after 2 min sonication, the vessel was warm to the touch and that the temperature increased with the duration of the experiment. It was concluded that the presence of As(V) in the soil extracts was due predominantly to oxidation during the sample pretreatment and so sonication was abandoned in favor of mechanical shaking.

Stability over 24 h

To study the oxidation, or other species interconversion, during the extraction procedure, solutions containing 1.0 mg l^{-1} of each species prepared in 0.10 mol l^{-1} H_3PO_4 or in 0.10 mol l^{-1} NaOH were shaken overnight and analyzed. The recoveries are

Table 3 The effect of solvent type, concentration, and sequence on the extraction of arsenic from soil containing 20 mg kg^{-1} As(III). Concentrations are in mg kg^{-1} as elemental arsenic in the original soil

			As(V)		As(III)		
Solvent	Technique	Time	Conc.	Total	Conc.	Total	As(III) + As(V) Total
10 mM H_3PO_4	Sonication	5 min	2.36	2.60	6.69	10.2	12.8
10 mM NaOH	Sonication	5 min	0.24		3.57		
100 mM H_3PO_4	Sonication	5 min	2.14	2.14	9.95	11.94	14.1
100 mM NaOH	Sonication	5 min	0.00		1.99		
100 mM NaOH	Sonication	5 min	0.30	0.99	11.0	14.8	15.8
100 mM H_3PO_4	Sonication	5 min	0.69		3.84		
100 mM H_3PO_4	Sonication in ice bath	5 min	2.91	2.91	8.21	12.4	15.3
100 mM NaOH	Sonication in ice bath	5 min	0.00		4.21		
100 mM NaOH	Sonication in ice bath	5 min	0.00	0.00	6.86	10.33	10.33
100 mM H_3PO_4	Sonication in ice bath	5 min	0.00		3.47		
100 mM H_3PO_4	Shaking	24 h	1.43	1.43	15.2	18.25	19.7
100 mM NaOH	Shaking	24 h	0.00		3.04		
100 mM NaOH	Shaking	24 h	0.00	0.00	13.7	16.27	16.27
100 mM H_3PO_4	Shaking	24 h	0.00		2.56		

Table 4 Stabilities of 1.0 mg l^{-1} As(III), DMA, MMA, and As(V) prepared in 0.10 mol l^{-1} H_3PO_4 and 0.10 mol l^{-1} NaOH, shaken for 24 h and analyzed by HPLC-HG-ICP-OES

	Concentration measured as a percentage of the original concentration (mean ± std dev, $n = 3$)			
Species and solvent	As(III)	DMA	MMA	As(V)
As(III) in H_3PO_4	99.5 ± 1.3	—	—	—
As(III) in NaOH	93.7 ± 1.6	—	—	4.6 ± 0.8
DMA in H_3PO_4	—	103.1 ± 2.2	—	1.6 ± 0.3
DMA in NaOH	—	106.2 ± 2.2	—	
MMA in H_3PO_4	—	—	110.2 ± 8.2	—
MMA in NaOH	3.3 ± 0.9	—	105.1 ± 4.8	—
As(V) in H_3PO_4	—	—	—	93.6 ± 4.7
As(V) in NaOH	—	—	—	104.8 ± 4.8

summarized in Table 4. It can be seen that no As(V) was observed when a standard of As(III) in H_3PO_4 was analyzed. However, a small amount of As(V) was detected when a standard of As(III) in NaOH was analyzed, corresponding to 5% of As(III) conversion to As(V). For the MMA standard in NaOH about 3% conversion to As(III) was observed.

Sequential extraction of all species

The chromatograms of the extracts are shown in Fig. 4 with peaks identified based on retention time matching with those of standards. It should be born in mind that the sensitivities for each compound are different due to the different efficiencies of

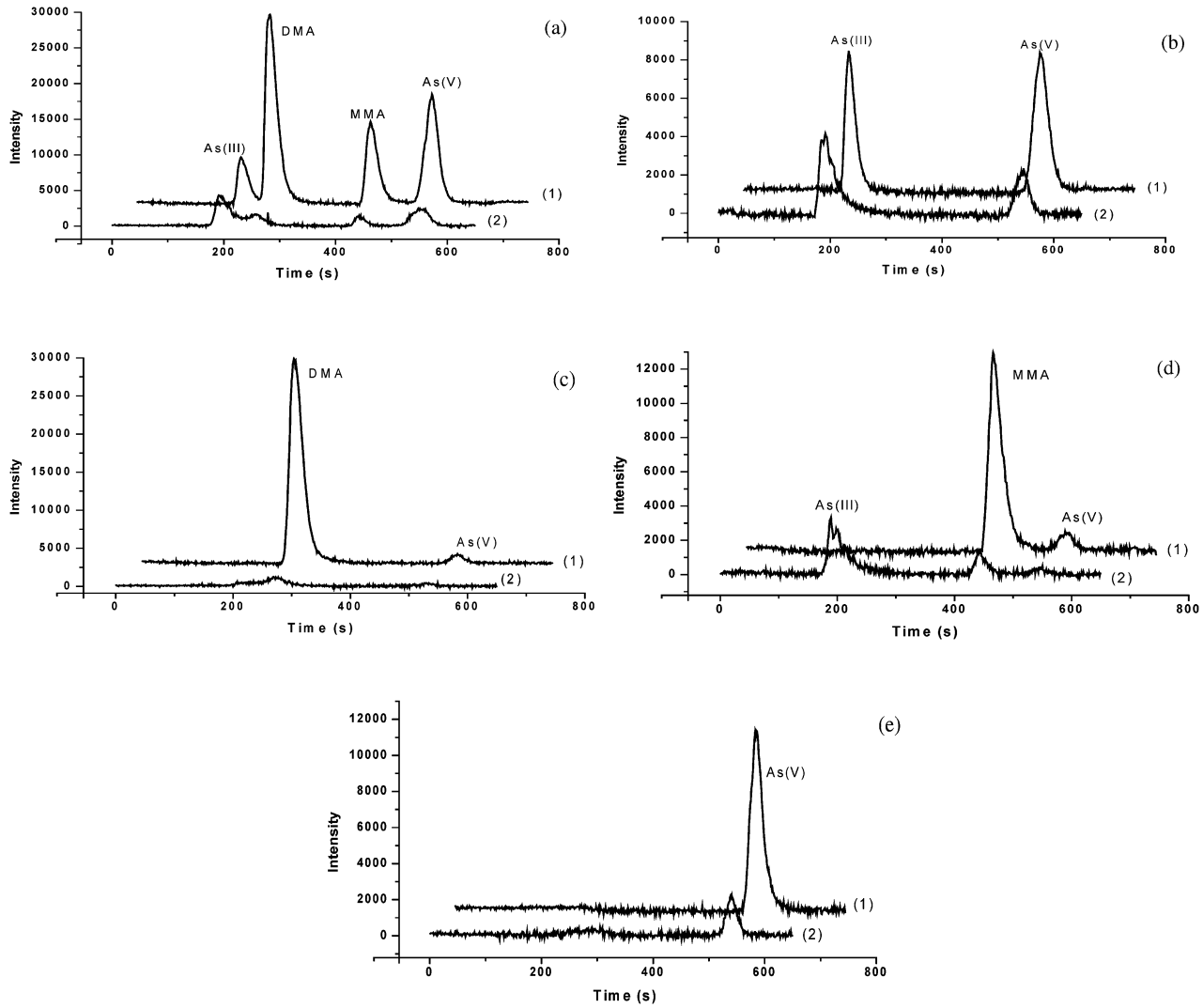

Fig. 4 Chromatograms for arsenic speciation by HPLC-HG-ICP-OES of sequential extracts of (a) mixed species–soil, (b) As(III)–soil, (c) DMA–soil, (d) MMA–soil, and (e) As(V)–soil; 1. 0.10 mol l^{-1} H_3PO_4; 2. 0.10 mol l^{-1} NaOH.

Table 5 Arsenic species concentrations in mg kg^{-1} based on sequential extraction with 0.10 mol l^{-1} phosphoric acid followed by 0.1 mol l^{-1} sodium hydroxide solution. Entries are mean values and standard deviations ($n = 3$)

	As(III)–soil			DMA–soil			MMA–soil			As(V)–soil			Mix–soil		
	H_3PO_4 Extract	NaOH Extract	Total	H_3PO_4 Extract	NaOH Extract	Total	H_3PO_4 Extract	NaOH Extract	Total	H_3PO_4 Extract	NaOH Extract	Total	H_3PO_4 Extract	NaOH Extract	Total
As(III)[a]	2.97	2.65	5.62	0.00	0.00	0.00	0.64	0.29	0.94	0.00	0.00	0.00	2.60	0.39	2.99
	±0.20	±0.22	±0.01	0.00	0.00	0.00	±1.11	±0.26	±1.1	0.00	0.00	0.00	±0.09	±0.00	±0.09
DMA[a]	0.00	0.00	0.00	17.92	1.24	19.17	0.00	0.00	0.00	0.00	0.00	0.00	15.81	2.70	18.51
	0.00	0.00	0.00	±1.43	±0.21	±1.47	0.00	0.00	0.00	0.00	0.00	0.00	±0.05	±0.63	±0.63
MMA[a]	0.00	0.00	0.00	0.00	0.00	0.00	17.87	1.58	19.45	0.00	0.00	0.00	15.63	1.39	17.02
	0.00	0.00	0.00	0.00	0.00	0.00	±0.45	±0.02	±0.45	0.00	0.00	0.00	±0.48	±0.09	±0.49
As(V)[a]	12.67	2.45	15.12	0.68	0.08	0.76	1.36	0.27	1.63	17.74	2.46	20.20	26.79	5.17	31.95
	±0.88	1.85	2.74	±0.60	±0.09	±0.66	±0.29	±0.06	±0.30	±0.14	±0.05	±0.14	±1.38	±0.07	±1.38
Total[a]	15.64	5.10	20.74	18.60	1.32	19.92	19.88	2.14	22.02	17.74	2.46	20.20	60.84	9.64	70.48
	±0.45	±0.93	±1.37	±0.78	±0.12	±0.80	±0.62	±0.13	±0.63	±0.07	±0.03	±0.07	±0.73	±0.32	±0.80

hydride generation, which is highest for As(III). The results are summarized in Table 5. Recoveries for total arsenic between 88% and 110% were obtained. About 75% of the As(III) was oxidized to As(V) in both the soil containing As(III) and the soil containing the mixtures. The fraction of arsenic extracted by H_3PO_4 constituted between 88% and 93% of the total extracts for DMA, MMA and As(V). However, phosphoric acid only extracted about 53% of the As(III) for which NaOH solution was still needed for complete extraction. The results indicate that some demethylation of the DMA and MMA species had occurred, though in the case of the DMA there was no detectable MMA, only As(V).

Conclusion

Sequential extraction with 0.10 mol l^{-1} H_3PO_4 and 0.10 mol l^{-1} NaOH by shaking for 24 h is an efficient procedure for extracting arsenic species from a soil to which the arsenic species have been added and are surface bound. The method oxidizes about 5% of the total As(III) concentration present in the sample, for which a correction could be made. In this study of a sterile soil stored in the dark, about 75% of the As(III) was oxidized to As(V) over a period of several weeks. Although sonication may accelerate the extraction of total arsenic, it is not suitable for arsenic speciation as it also accelerates the oxidization of As(III) to As(V). The procedure has not been evaluated for a soil with higher organic content, for which there may be problems due to the higher concentration of humic material in the alkaline extract. It is possible that the procedure has broader applicability, such as to the determination of arsenic species in foodstuffs, especially rice. These possibilities are the subject of on-going further work.

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