APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2002; **16**: 347–354
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.311

Comparison of extraction procedures for arsenic speciation in environmental solid reference materials by high-performance liquid chromatography–hydride generation-atomic fluorescence spectroscopy

M. Montperrus[1], Y. Bohari[2], M. Bueno[1], A. Astruc[1]* and M. Astruc[1]

[1]L.C.A.B.I.E. UMR CNRS 5034, Université de Pau et des Pays de l'Adour, Avenue de l'Université, 64000 Pau, France
[2]Department of Chemistry, Mulawarman University, Samarinda, Indonesia

Received 7 January 2002; Accepted 19 March 2002

Water and 'soft' extractions (hydroxylammonium hydrochloride, ammonium oxalate and orthophosphoric acid) have been studied and applied to the determination of arsenic species (arsenite, arsenate, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)) in three environmental solid reference materials (river sediment, agricultural soil, sewage sludge) certified for their total arsenic content. The analytical method used was ion exchange liquid chromatography coupled on-line to atomic fluorescence spectroscopy through hydride generation. Very low detection limits for arsenic were obtained, ranging from 0.02 to 0.04 mg kg^{-1} for all species in all matrices studied. Orthophosphoric acid is the best extractant for sediment (mixed origin) and sludge samples (recent origin) but not for the old formation soil sample, from which arsenic is extracted well only by oxalate. Both inorganic forms (As(III) and As(V)) are significant in all samples, As(V) species being predominant. Moreover, organic forms are found in water extracts of all samples and are more important in the sludge sample. These organic forms are also present in the 'soft' extracts of sludge. Microwave-assisted extraction appears to minimize the risk of a redox interconversion of inorganic arsenic forms. This study points out the necessity of combining direct and sequential extraction procedures to allow for initial arsenic speciation and to elucidate the different mineralogical phases–species associations. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: arsenic speciation; liquid chromatography; atomic fluorescence spectroscopy; environmental reference materials samples; extraction

INTRODUCTION

Arsenic is present at high concentration levels in soils and sediments in a very large number of places in the world. This is due to either its geochemical origin or to mining activities and industrial production, mainly for agricultural uses as insecticides, herbicides and fungicides.[1] Moreover, contamination may occur by disposal of industrial, municipal and animal wastes. In non-contaminated soils the arsenic contents are in the 1 to 40 mg kg^{-1} range, but arsenic concentrations higher than 20 g kg^{-1} may be measured in old industrial or mining sites.[2–4]

Arsenic toxicity is strictly correlated to its chemical forms, inorganic arsenic species being the most toxic ones; methylated species present intermediate toxicity and larger biomolecules are non-toxic.[5]

Arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are the most often encountered forms, and also the most often studied in soils and sediments owing to their ability to dissolve in water under environmental conditions. The existence of these forms depends upon parameters such as redox potential, pH[6] and the intensity of biogeochemical processes that affect arsenic mobilization.[7] Many minerals containing arsenic (for

*Correspondence to: A. Astruc, L.C.A.B.I.E. UMR CNRS 5034, Université de Pau et des Pays de l'Adour, Avenue de l'Université, 64000 Pau, France.
E-mail: annette.astruc@univ-pau.fr
Contract/grant sponsor: ECOS.
Contract/grant sponsor: Région Aquitaine.
Contract/grant sponsor: SFERE.

example, arsenopyrite) may be present in soils without immediate environmental risk because their water solubility is very low, except in the long term in special conditions.[8] Biogeochemical processes may, however, convert these minerals to more mobile forms.

With direct speciation analysis in the solid phase still not being possible, the first step of all procedures is an extraction step, called hereafter a 'soft' extraction procedure, which must separate the analytes from the matrix, eliminate or reduce the interferences of other components and concentrate the analytes up to detectable concentration levels without losses or contamination and also without changes in speciation.[9]

Pantsar-Kallio and Manninen[10] demonstrated that optimal total arsenic extraction yields from soils were obtained in very acidic (pH ~1) or alkaline (pH ~13) conditions and that As(III) stability was lower at high pH values. Several authors have already demonstrated that microwave-assisted orthophosphoric acid extraction of arsenic species from soils and sediments is efficient.[11–13] The most extensive study[13] was applied only to a spiked sediment sample ([As] = 200 mg kg^{-1}).

Gomez-Ariza *et al.*[14] concluded that hydroxylammonium hydrochloride solutions constitute a suitable reagent for arsenic extraction and speciation in sediments with high content of iron oxides.

Gleyzes *et al.*[15] elaborated a simplified arsenic-fractionation procedure and evaluated the so-called 'arsenic fraction extractable under moderately reducing and complexing conditions' using an oxalate–oxalic acid solution.

In this study we compared water extraction and different 'soft' microwave-assisted chemical extractions using anion-exchange high-performance liquid chromatography (HPLC), hyphenated through on-line hydride generation to atomic fluorescence spectrometry (HG-AFS) as the analytical method. The very high sensitivity of the detector for arsenic determination[16] makes it possible to investigate the effects of variations of extractant concentration, even with samples with a quite low total arsenic content.

Up to now, there has been no environmental solid certified reference material (CRM) for arsenic speciation, therefore, we analysed reference materials available world wide and certified for their total arsenic content in order to allow intercomparisons with other laboratories.

EXPERIMENTAL

Standard solutions and reagents

Arsenic standards used were of the highest purity available, i.e. analytical grade, except for the orthophosphoric acid extractant, which was Merck Suprapur.

Stock solutions ([As] = 1000 mg l^{-1}) of arsenic species were prepared by dissolving $NaAsO_2$ (Merck), $Na_2HAsO_4{\cdot}7H_2O$ (Prolabo), $CH_3AsO(ONa)_2{\cdot}6H_2O$ (Carlo Erba), $(CH_3)_2AsO_2Na{\cdot}3H_2O$ (Stream Chemicals) in water Milli-Q Gradient + A10 high-quality (18 MΩ, TOC <4 μg l^{-1}) (Millipore). These standard solutions were stored at 4 °C in the dark and their stability frequently checked. Arsenite solution was kept for 1 month only, to prevent eventual transformation, instead of the 6 months for the others. Intermediate standard solutions ([As] = 10 mg l^{-1}) were prepared daily and used directly for the preparation of appropriate working standard solutions. Working standard solutions were tested every day in the quality control of the analytical procedure.

Phosphate buffers used as HPLC mobile phases were prepared from $(NH_4)_2HPO_4$ (Merck) and $NH_4H_2PO_4$ (Carlo Erba) aqueous solutions. The pH was adjusted by addition of an aqueous NH_3 solution (Merck). The chromatographic mobile phase was continuously filtered through a 0.45 μm membrane.

A 2.5% m/v $NaBH_4$ (Fluka), stabilized with 1% m/v NaOH (Merck) solution was prepared immediately prior to the experiment.

H_3PO_4 was purchased from Merck, NH_2OH–HCl from Prolabo, and $(COOH)_2{\cdot}2H_2O$ and $(NH_4)_2C_2O_4{\cdot}H_2O$ from Carlo Erba.

All containers were previously decontaminated by soaking for several days in a 10% HNO_3 solution and rinsed with ultra-pure water before use.

Apparatus

The HPLC system consisted of a Varian 5000 gradient solvent delivery system equipped with a 100 μl injection loop (Interchim) and a 25 cm × 4.1 mm i.d. (Hamilton, PRP-X100) anion-exchange column at room temperature.

The outlet of the column was connected to an on-line continuous flow hydride generation system, model ES120S (Spectra France, Pau, France). A Labcraft peristaltic pump was used for the successive additions of HCl and $NaBH_4$ solutions.

The Excalibur atomic fluorescence detector manufactured by PSAnalytical (10033) was equipped with a boosted discharge hollow cathode lamp (Photron) and the signal output was recorded with a computer using Borwin chromatographic software (JMBS, Grenoble, France). A supplementary hydrogen input, direct to the argon–hydrogen diffusion flame, was added to the original Excalibur design in order to stabilize the flame, and thus to smooth the base-line signal, whereas in the original PSA device the flame was supplied only by the excess hydrogen produced during sodium borohydride decomposition.[16]

The open focused microwave oven used in the extraction step was a Prolabo Microdigest 301 system (power 0 to 200 W). The centrifugation was carried out with a Hettich centrifuge, model Universal 16.

Analytical procedure

A 100 μl volume of standard solution (eventually diluted)

Table 1. HPLC–HG-AFS experimental conditions and performance

HPLC procedure	
Anion exchange column	Hamilton PRP-X100 (250 mm × 4.1 mm i.d., 10 μm particle size)
Mobile phase	Sol A: ammonium phosphate ($NH_4H_2PO_4$/$(NH_4)_2HPO_4$) 5 mmol l^{-1} pH 4.8
	Sol B: ammonium phosphate ($NH_4H_2PO_4$/$(NH_4)_2HPO_4$) 30 mmol l^{-1} pH 8.0
Injected volume	100 μl
Flow rate	1 ml min^{-1}
Gradient elution	0 to 4.1 min, Sol A
	4.1 to 10.1 min, Sol B
	10.1 to 20.0 min, Sol A
HG-AFS procedure	
Acid solution	3.0 mol l^{-1}; 0.35 ml min^{-1}
Reducing agent	2.5% (w/v) $NaBH_4$ in 1% (w/v) NaOH; 0.35 ml min^{-1}
Main argon flow rate	100 ml min^{-1}
Auxiliary argon flow rate	300 ml min^{-1}
Hydrogen flow rate	30 ml min^{-1}
Primary current	27.5 mA
Boost current	35.0 mA

Performance	As(III)	As(V)	MMA	DMA
Linearity range (ng As)	0.005–20	0.006–40	0.005–20	0.007–40
Reproducibility (%)[a]	5	4	4	5
Relative detection limit (ng (As) ml^{-1})	0.05	0.07	0.05	0.06
LOD[b] in solid materials (mg (As) kg^{-1})				
Soil SRM 2709	0.02	0.03	0.02	0.02
Sediment CRM 320	0.02	0.02	0.02	0.02
Sludge CRM 007-040	0.04	0.04	0.04	0.04

[a] Relative standard deviation of ten analyses.
[b] Calculated as three times standard deviation of 20 blanks/slope of calibration curve.

sample was injected into the HPLC system. The elution was achieved with a gradient program established using two ammonium phosphate buffers: (Sol A) 5 mM, pH 4.7 and (Sol B) 30 mM, pH 8.0. From 0 to 4.1 min Sol A was pumped, then Sol B from 4.1 to 10.1 min. Sol A was pumped again from 10.1 to 20.0 min in order to equilibrate the column before the following analysis.

This elution procedure has been optimized from previous work,[16,17] with special attention being paid to obtaining the elution of As(III) species out of the dead volume and obtaining a good resolution between the different peaks.

HCl and $NaBH_4$ solutions were continuously added to the column effluent by the peristaltic pump via two successive T-pieces, assuming the reduction of As(V) species to As(III) and the generation of hydrides corresponding to As(III), DMA and MMA. These volatile arsines were purged from the liquid in a Spectra France gas–liquid separator by an argon flux and carried to the detector via a Permapure hygroscopic membrane drying tube. In these conditions the four arsenic species were eluted in the order As(III), DMA, MMA, As(V), with elution times of 2.9 min, 5.9 min, 8.7 min and 12.8 min respectively.

Analytical performances

General analytical performances are summarized in Table 1. Further details are published elsewhere.[16]

Standard solutions

Linearity is good up to 200 μg l^{-1} for the four arsenic species considered.

Reproducibility test at the 5 μg l^{-1} level leads to RSD values varying between 4 and 5%. Arsenic detection limits evaluated following the IUPAC definition are between 5 and 7 pg (mass limit of detection (LOD)) or 0.05 and 0.07 μg l^{-1} (concentration LOD).

Environmental solids

Our experimental conditions led to an arsenic LOD in the range 0.02 to 0.04 mg kg^{-1} for all species in the three CRM matrices considered. Compared with its Thomas *et al.*[12] LOD values, the improvement factor was from 40 to 80. This high sensitivity allows one either to analyse samples with a low arsenic content or to dilute arsenic-rich extracts in order to minimize eventual matrix effects on the detection or even during the chromatographic separation.[18]

Samples and extractants

The three CRMs were chosen for their certified total arsenic content, because they represent the various kinds of environmental solids, and for their different origins.

The first is a soil (San Joaquim SRM 2709; NIST (USA), NIST Gaithersburg) coming from an agricultural region of California; its total arsenic content is 17.7 ± 0.8 mg kg^{-1}. The second is an aerobic river sediment polluted by industrial activities (CRM 320; BCR (EU), IRMM Geel) containing 76.7 ± 3.4 mg kg^{-1}. The third is a digested sewage sludge coming from a publicly owned sewage treatment works that is representative of a residential area with industrial influence (CRM 007-040; RT (USA), Promochem GmbH) and contains 5.74 ± 0.85 mg kg^{-1}.

'Soft' extractants were selected in order to extract mobile arsenic species from solid matrices without modifying them: viz., water, hydroxylammonium hydrochloride (0.1 mol l^{-1}) ammonium oxalate (0.2 mol l^{-1}) and orthophosphoric acid (0.3 mol l^{-1}).

Extraction procedure

Water extraction

The water extraction was realized according to the AFNOR X 31–210 method. A mass of 0.5 g of dry solid material was mixed with 5 ml of ultra-pure water in a polypropylene centrifuge tube. The solution was degassed for 20 min with an argon flow to eliminate oxygen. The tube was then shaken for 24 h on a stirring table, centrifuged at 3400 rpm for 15 min and the supernatant filtered through a 0.2 μm filter before chromatographic analysis.

'Soft' extractions

The extraction conditions applied to the three CRM were based on those described by Thomas *et al.*,[12] which were found convenient in our laboratory after detailed investigations of the influence of sample mass/extractant volume ratio and of microwave conditions.

A mass of *ca* 0.15 or 0.3 g of dry solid material was introduced in a digestion tube with 25 or 50 ml of extracting solution and submitted to microwave irradiation at 40 W for 20 min. After cooling, the mixture obtained was transferred with the corresponding rinse water to a centrifuge tube. After 20 min at 3400 rpm all the supernatant was diluted in a 50 or 100 ml flask and filtered through a 0.2 μm filter before chromatographic analysis. Secondary dilutions of the extracts before analysis by factors of 10 to 50, sometimes more, were often used. The possibility of replacing centrifugation by filtration, which requires a supplementary rinsing step of the filter before dilution in a known volume, and thus possible further evolution of arsenic speciation, has been tested. Two results were the same, considering the reproducibility of the analytical method.

The overall extraction procedures were first tested by treating standard solutions of the four arsenic species considered, in order to test for any eventual inter-transformations of species that might occur, especially between As(III) and As(V). No arsenic species transformation was detected, even when these solutions were stored over several hours; the same conclusions have already been reached by previous workers.[13] This indicates that the arsenic species considered are stable under the conditions of the various 'soft' extraction procedures applied to standard solutions (i.e. not in the presence of natural solid extracts).

RESULTS AND DISCUSSION

All the yield values presented below are the ratios of total extracted arsenic (evaluated as the sum of the various arsenic species) to the certified values.

Water extraction

As expected, the percentages of total arsenic extracted by water from the three CRMs studied are low, the best being obtained when extracting sludge. This is probably due to the recent origin of the sludge material compared with the other two (which could have been exposed before, over a long period, to various natural water extraction processes, e.g. rain, watering, flooding or river flow).

Inorganic arsenic is present in both soil and sediment extracts, with similar proportions of arsenite and arsenate in the sediment, arsenate being preponderant in the soil extract. The soil considered in this study was either a more oxidized medium than the river sediment or had been modified during the CRM preparation procedure.

In the sludge the four arsenic species are present, probably due to a high content of organic matter able to favour the conversion of inorganic arsenic into organic forms via (bio)methylation processes.

Solid-water exchange of arsenic species

Known quantities of the four arsenic species were added simultaneously to extraction flasks containing already weighed amounts of the analysed solids at the same time as the extracting water. This procedure does not allow a long equilibration time (only over the 24 h of the test), and added arsenic compounds should, of course, be more easily extractable than compounds naturally present.[9] These experiments were run in triplicate. Concentration increments were chosen so as to be of the same order of magnitude as the concentrations obtained by the direct extractions, on raw materials.

Total arsenic addition recoveries are rather low (30–42%), whatever the material studied (Table 2).

Added As(III) recovery is in the range 25–30% for all matrices; very little As(V) is recovered (less than 15%, and no recovery at all for the sludge sample); MMA recovery from soil and sediment is low (10–13%), but it reaches 65% for the sludge sample; DMA is the only species that does not seem to establish strong links with the solid matrices, water

Table 2. Water extraction of the three CRM and recovery of spiked arsenic species

		Arsenic species in extracts (%)			
	TAE[a] (%)	As(III)	As(V)	DMA	MMA
Soil	0.6 ± 0.1	0.07 (11)	0.40 (67)	0.03 (5)	0.10 (17)
Sediment	0.2 ± 0.1	0.08 (42)	0.09 (46)	0.02 (9)	0.01 (2)
Sludge	9.7 ± 0.1	1.3 (13)	2.9 (30)	2.5 (26)	3 (31)
			Addition recovery[b]		
Soil	40 ± 2	30 [4]	14 [8]	110 [4]	13 [4]
Sediment	30 ± 2	29 [10]	10 [10]	80 [5]	10 [5]
Sludge	42 ± 3	25 [10]	nd [20]	90 [10]	65 [20]

[a] Total arsenic extracted (TAE) is the ratio of arsenic concentration extracted to the certified total arsenic concentration in the sample; relative percentage of arsenic species to TAE in parentheses.
[b] These experiments were run in triplicate; addition value expressed in μg (As) kg^{-1} (triplicate) in square brackets; nd: not determined.

extraction yields of added amounts being close to quantitative (80–110%).

During the 24 h shaking involved in the water extraction procedure, it is likely that most species added are adsorbed onto samples, for example onto iron and/or aluminium hydroxides contained in soil and sediment or organic matter contained in sludge, either as introduced or following a species conversion such as As(III) ⇌ As(V). In these approximately neutral conditions all arsenic species are anionic except As(III), so the differences in behaviour apparent in Table 2 cannot be explained on the basis of charges alone. The exceptionally high mobility of DMA implies that it is almost not sorbed by any of the three solid matrices tested. The very low mobility of As(V) implies a strong sorption. The well-known high affinity of As(V) for iron and manganese oxides surfaces is a good explanation for soil and sediment samples; the even stronger sorption by the sludge sample is less easy to explain. A possible hypothesis could be a reduction of As(V) to As(III) by the organic-rich matrix followed by hydrophobic sorption of the neutral As(III) species to the abundant solid organic matter.

'Soft' extractions

Three soft extractants were chosen from the literature and from preliminary experiments as being able to dissolve the better part of the arsenic species without modifying them: viz., A (0.3 mol l^{-1} orthophosphoric acid); B (0.1 mol l^{-1} hydroxylammonium hydrochloride); C (0.2 mol l^{-1} di-ammonium oxalate).

Efficiency of 'soft' extractions as regards total arsenic

The total arsenic extraction yields obtained (Table 3) depend upon both the nature of the extractant used and that of the sample studied.

Total arsenic extraction yields of soil samples are low using orthophosphoric acid or hydroxylamine extractants, but the ammonium oxalate buffer (known to form complexes with the Fe(III) cation and to dissolve crystalline iron oxides in classical sequential extraction schemes[15,19,20]) is much more efficient (82 ± 3%). In this agricultural soil sample the mineralogical matrix is probably of very ancient formation and most of its components are weathered crystallized minerals. It may be supposed that arsenic species in this matrix are for the better part linked to (or even integrated in) crystalline iron and manganese oxy-hydroxides.

The aerobic river sediment sample (BCR 320) also contains manganese oxides (Mn 0.08%), together with soluble or non-soluble iron compounds (Fe 4.5%); but arsenic species appear to be much more weakly linked to the solid matrix (probably sorbed to surfaces), as the three extractants studied produce rather good yields. The efficiency of phosphate ions in releasing arsenic from environmental sediments is essentially based on the ion-exchange surface reaction between phosphate and arsenate ions.[13]

Hydroxylamine extraction of sediment BCR 320 sample followed by HPLC–HG-atomic absorption spectroscopic analysis of the extracts has been previously conducted by Gomez-Ariza *et al.*,[14] who obtained a lower extraction yield with a somewhat different extraction procedure (8 h, 95 °C) even after repetitive (five to eight) extractions.

This same reference material has also been studied by Thomas *et al.*[12] using extraction conditions very similar to those used here (1 mol l^{-1} H_3PO_4 instead of 0.3 mol l^{-1}), but

Table 3. 'Soft' extractions: total arsenic extraction yields (percentage of total arsenic content of the solid) using A, B and C

Extracting solution	A	B	C
Soil	37 ± 3	10 ± 2	82 ± 3
Sediment	94 ± 3 *(54)*[a]	73 ± 2 *(67)*[b]	80 ± 2
Sludge	93 ± 4	79 ± 3	–

[a] Ref. 12.
[b] Ref. 14.

Table 4. Influence of the nature of the extractant on the determination of arsenic speciation in different matrices (percentage of CRM total arsenic content)[a]

	As(III)	DMA	MMA	As(V)
Soil				
A	2 (5)	–	–	38 (95)
B	2 (20)	–	–	8 (80)
C	3 (4)	–	–	77 (96)
Sediment				
A	7 (7)			89 (93)
	3 (6)[b]			*51 (94)*[b]
B	17 (23)			56 (77)
	3 (4)[c]			*64 (96)*[c]
C	11 (14)			68 (86)
Sludge				
A	21 (22)	4 (4)	6 (6)	65 (66)
B	12 (15)	2 (3)	5 (6)	60 (75)

[a] A: phosphoric acid (0.3 mol l^{-1}); B: hydroxylammonium hydrochloride (0.1 mol l^{-1}); C: ammonium oxalate (0.2 mol l^{-1}); relative percentage of arsenic species to TAE given in parentheses.
[b] v (*w*) Ref. 12 values.
[c] v (*w*) Ref. 14 values.

followed by an HPLC–inductively coupled plasma mass spectrometric analysis of extracts. These authors obtained a much lower phosphoric acid extraction yield, probably due to working conditions too close to their quantification limits and/or suffering from matrix interferences.

The efficiency of the extraction of arsenic from the sewage sludge CRM is greatest efficient with orthophosphoric acid, adequate with the hydroxylammonium hydrochloride solution, but arsenic is not extracted at all by the oxalate buffer from this matrix. In this freshly formed organic-rich material it may be reasonably supposed that the majority of the arsenic species are linked to organic substances and none to crystalline metal oxides.

Influence of the nature of extractant on arsenic speciation

In the three environmental solid reference materials studied, inorganic arsenic species (mainly arsenate), are the major forms, methylated forms appearing only as minor components in the sewage sludge sample.

The repartition of arsenic species evaluated with the 'soft' extractants tested (Table 4) is quite different from that presented above using water extraction (Table 2). In particular, noticeable percentages of methylated species are found only in the sewage sludge extracts (10% of total arsenic content) and none at all in those of soil and sediment samples, whereas the proportions of MMA and DMA measured in soil and sediment water extracts were not negligible (0.1% and 0.02% of their total arsenic contents

Table 5. Influence of the extraction time on arsenic speciation of sediment CRM (percentage of CRM total arsenic content)

Extractant	Extraction time (min)	As(III) (%)	As(V) (%)	TAE (%)
B	20	17	56	73
	40	17	54	71
		3[a]	64[a]	67[a]
C	10	12	62	74
	20	8	67	75
	40	–	70	70

[a] Ref. 14 results; extraction conditions: 8 h, 95 °C, occasional shaking.

respectively). In fact, the increases in the extraction yields of inorganic arsenic species from soil and sediment using extractants A or B are so high (a factor of *ca* 20) that the concentrations of the methylated forms of arsenic in the solutions analysed (after suitable dilutions) become lower than the detection limits.

Both extractants A and B dissolve the same amounts of DMA (2–4% of total arsenic) and MMA (5–6% of total arsenic) from the sludge sample.

Soil sample. The percentage of total arsenic extracted from the soil sample as As(III) is very low (2–3%) for the three extractants, whereas the percentage extracted as As(V) varies widely (from 8% with hydroxylammonium hydrochloride to 77% with ammonium oxalate). It appears, therefore, that no conversion of arsenic species occurs in any of these extracting operations. The different extracting reagents mobilize widely variable proportions of total arsenic. Ammonium oxalate, well known to dissolve crystallized iron and manganese (oxy)hydroxides, which contain quite a large proportion of the arsenic content of this material, is the best extracting reagent.

To recap, the As(III) content of this material may be quite reliably evaluated to *ca* 2–3% of total arsenic; the evaluation of the As(V) content, on the contrary, depends greatly on the mineralogical phases dissolved during the 'soft' extraction.

Sediment sample. The same conclusions do not hold when considering data obtained for the river sediment; the percentage of As(III) determined varies from 7 to 17% of total arsenic, whereas that of As(V) varies from 89 to 56%. The occurrence of some redox interconversion of species in the presence of natural solid extracts is necessary to explain these data.

This same CRM has already been examined by two other authors using slightly different extraction conditions and a detailed examination of the results may afford better understanding.

Comparing arsenic speciation evaluated following hy-

droxylamine extraction using both our fast microwave procedure and the procedure of Gomez Ariza *et al.*,[14] who use occasional shaking for 8 h at 95 °C (Table 4), demonstrates that our procedure extracts some more arsenic (73% of the total instead of 67%) and that the proportion of As(III) is much higher with our 'fast' procedure (17% instead of 3%). Three possibilities must be considered, taking into account both the reducing property of hydroxylamine and the possible oxidation by atmospheric oxygen in the presence of soil extracts:

- all the supplementary arsenic extracted with our procedure is really As(III) dissolved due to the efficiency of the microwave-assisted process;
- supplementary As(V) extracted in our procedure is simultaneously reduced to As(III) by hydroxylamine for the same reason;
- the lengthy Gomez Ariza *et al.*[14] extraction procedure allows a significant As(III) oxidation by atmospheric oxygen.

Extraction by ammonium oxalate, which is also a reducing agent, dissolves some more total arsenic with a lesser proportion of As(III).

A comparison can also be made between arsenic speciation in this same sediment determined following fast microwave-assisted phosphoric acid extraction using either 0.3 mol l^{-1} (this work) or 1.0 mol l^{-1} acid concentrations.[12] The same proportions of As(III) and As(V) were found even if the total arsenic extraction yield of the present work is much higher (96% versus 54%).

It appears here that the conditions of extraction have a non-negligible impact on the evaluation of inorganic arsenic species initially present in this river sediment: the As(III) content determined varies from 3 to 17% of total arsenic and As(V) from 51 to 89%.

Sludge sample. More arsenic is extracted by phosphoric acid than by hydroxylamine (96% and 79%) and the increase concerns both As(III) and As(V). The proportions of MMA (2% and 4%) and DMA (6% and 5%) do not vary much. Considering the very high extraction yield obtained with phosphoric acid, it may be concluded that there is no other arsenic species present at a significant level in this sludge sample.

Influence of extraction time on the determination of arsenic speciation

Using hydroxylamine (extractant B), the total arsenic extracted does not vary significantly with microwave irradiation time; neither does As(III), which remains much higher than that found in Ref. 14. This confirms that a noticeable oxidation by atmospheric oxygen occurs during the 8 h of their extraction process, much more than the reverse reduction by hydroxylamine. This oxidation appears negligible during the 20–40 min of our procedure. A supplementary proof has been obtained by adding ascorbic acid to the extracting solution; no modification of arsenic speciation was evidenced.

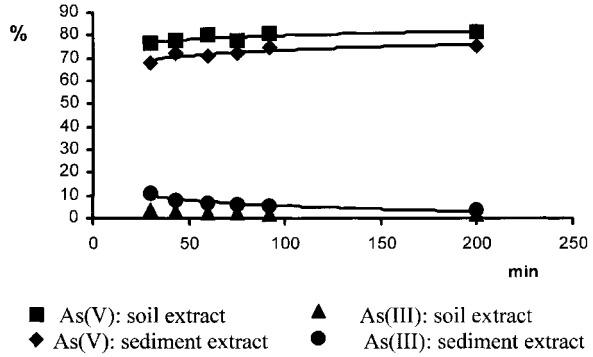

Figure 1. Stability of soil and sediment oxalate buffer (C) extracts (relative percentage of arsenic species to total arsenic extracted).

The behaviour in the presence of oxalate is different. There is a slight diminution of total arsenic extracted with microwave irradiation time. Moreover, the As(III) content decreases to zero. This indicates that the larger amounts of iron and manganese oxides dissolved by this reactant induce a much faster air oxidation of As(III) (perhaps involving a catalytic process).

Stability of extracts

The stability of As(III) in the phosphoric acid extracts has already been studied:[18] neutralization or dilution ensure a good stability for several hours.

Further experiments were performed in order to elucidate the problem of As(III) stability during the conservation of oxalate extracts of sediment and soil samples. Each extract prepared was analysed from 30 to 250 min after the end of extraction procedure. We can see in Fig. 1 that As(III) oxidation to As(V) after extraction is a slow process (the rate of which depends on the nature of the sample being analysed). The relative As(III)/total extracted arsenic ratio decreases from 14% to 5% (sediment) or from 4% to 2% (soil) in 4 h. Once again, extract dilution may slow down these evolutions.

On the other hand, we do not observe any loss of arsenic during these experiments. All this confirms the hypothesis presented above, of the role played by some sample constituents (dissolved during the extraction) probably catalysing As(III) oxidation by dissolved oxygen.

CONCLUSIONS

The first and most important conclusion is that the determination of the speciation of elements such as arsenic

in environmental solids is still a very difficult task, because extraction procedures need to be designed so as to allow:

- respect for the chemical nature of the various arsenic species initially present in the solid;
- elucidation of their association with the various mineralogical phases of the sample (fractionation).

This task will be completed by combining the type of research work presented here together with others already existing,[15] which up to now have been separated in the literature.

The four species studied here, As(III), As(V), MMA and DMA, have all been found in the water extracts of all samples. The organic forms are important only in the sludge sample; both As(III) and As(V) are significant in all samples, and As(V) predominates.

Several extraction procedures using different extractants and different conditions have been considered and applied to three CRMs of soil, sediment and sewage sludge.

Microwave-assisted extraction appears to be very convenient, owing to the rapidity of the procedure, which moreover certainly minimizes the risks of redox interconversion of inorganic arsenic forms. However, we cannot ascertain from all these results that no oxidation at all of genuine arsenic species took place during the 20 min of the extraction step itself, and the As(III) concentrations reported here should be considered as minimum values.

In the mineralogic matrix of an old formation soil sample, extracted well only by oxalate, arsenic seems to be strongly linked to a low reactive phase, such as metallic oxyhydroxides.

On the other hand, in freshly formed sewage sludge, orthophosphoric acid extracts arsenic well, whether it is linked to exchangeable forms or acid-soluble solids.

Finally, the sediment sample is extracted well with the three 'soft' extractants owing to its mixed origin.

Acknowledgements

The authors would like to thank ECOS (Scientific Cooperation between France and Chile) Action C96E04, Région Aquitaine and SFERE (Scientific Cooperation with Indonesia).

REFERENCES

1. Azcue JM and Nriagu JO. Arsenic: historical perspectives. In *Arsenic in the Environment*, Nriagu JO (ed.). Wiley: Chichester, 1994; 1–15.
2. Morita M and Edmonds JS. *Pure Appl. Chem.* 1992; **64**: 575.
3. Gleyzes C. PhD Thesis, Université of Pau, 1999; US17299.
4. Kavanagh PJ, Farago ME, Thornton I and Braman RS. *Chem. Spec. Bioavailab.* 1997; **9**: 77.
5. Craig PJ. Organic Group VI elements in the environment. In *Organometallic Compounds in the Environment*, Longman: 1986; 254–278.
6. Masscheleyn PH, Delaune RD and Patrick Jr WH. *Environ. Sci. Technol.* 1991; **25**: 1414.
7. Livesey NT and Huang PM. *Soil Sci.* 1981; **131**: 88.
8. Rochette EA, Li GC and Fendorf SE. *Soil Sci. Soc. Am. Proc.* 1998; **62**: 1530.
9. Morabito R. *Fresenius' J. Anal. Chem.* 1995; **351**: 378.
10. Pantzar-Kallio M and Manninen PKG. *Sci. Total Environ.* 1997; **204**: 193.
11. Amran B, Lagarde F, Leroy MJF, Lamotte A, Demesmay C, Ollé M, Albert M, Rauret G and López-Sánchez JF. Arsenic speciation in environmental matrices. In *Quality Assurance for Environmental Analysis*, Quevauviller Ph, Maier E, Griepink B (eds). Elsevier: Amsterdam, 1995; 285–305.
12. Thomas P, Finnie JK and Williams JG. *J. Anal. At. Spectrom.* 1997; **12**: 1367.
13. Demesmay C and Olle M. *Fresenius' J. Anal. Chem.* 1997; **357**: 1116.
14. Gomez-Ariza JL, Sanchez-Rodas D, Giraldez I and Morales E. *Talanta* 2000; **51**: 257.
15. Gleyzes C, Tellier S and Astruc M. In *Methodology for soil and sediment fractionation studies – Single and sequential procedures*, Quevauviller Ph (ed.). Royal Society of Chemistry: 2002 (in press).
16. Bohari Y, Astruc A, Astruc M and Cloud J. *J. Anal. Atom. Spectrom.* 2001; **16**: 774.
17. Bohari Y. *PhD Thesis*, Université of Pau, 2001; UIPAUU3008.
18. Vergara Gallardo M, Bohari Y, Astruc A, Potin-Gautier M and Astruc M. *Anal. Chim. Acta* 2001; **441**: 257.
19. Belzile N and Tessier A. *Geochim. Cosmochim. Acta* 1990; **54**: 103.
20. Sadiq M. *Water Air Soil Pollut.* 1997; **93**: 117.

