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New preservation method for inorganic arsenic speciation in acid mine drainage samples

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Abstract

A new preservation method has been proposed for the speciation of As(III) and As(V) in acid mine drainage (AMD) samples, characterised by low pH and high metallic content. Samples were taken from a polymetallic sulphides mining area in the province of Huelva (SW Spain), under exploitation until the 1960s for its Cu, Pb and Zn sulphides. The abandoned mine works and the numerous waste rocks heaps produce AMD with high As content, an aqueous pollution source for the nearby streams. Short-term (from few hours to 1 week) preservation of the two inorganic arsenic species was studied, trying different containers (polyethylene, glass), presence or absence of light, temperatures (ambient, refrigerated, frozen), preserving agents and procedures (EDTA, HCl or AcH acids, cation-exchange resin). The speciation results obtained by liquid chromatography-hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS) indicated a rapid conversion of the samples with most of the preservation procedures reported in the literature after 3 h after sample collection. A promising method for arsenic preservation has been developed in this work, which maintains the arsenic species distribution in the original samples for a longer time. It consists in the use of opaque glass containers, acidification of the samples with HCl and in situ cleanup with cationic exchange resin, which allowed to preserve the samples for As speciation for at least 48 h.

Keywords: Arsenic; Speciation; AMD; Preservation; Cationic exchange; AFS

1. Introduction

Arsenic is an element ubiquitous in nature that can form numerous species, each with distinct occurrence, mobility, geochemical transformation and potential toxicity. Chemical speciation of arsenic in natural waters encompasses the determination of the inorganic oxyanions arsenite (As(III)) and arsenate (As(V)), and alkylated species (mainly monomethylarsonic (MMA) and dimethylarsinic (DMA) ions), these later being the result of biological activity. As(III) and As(V) represent the main species usually found in natural waters, As(III) being more toxic than As(V) [1]. The predominance of one or other in natural water depends on parameters such as pH, redox potential and the presence of other species in solution. On the other hand, the presence of the less toxic methylated species is highly variable, sometimes below detection limits, depending on the parameters that affect their biological production (e.g. pH, temperature, nutrients, turbidity) [2].

Acid mine drainage (AMD) represents a form of water pollution characterised by high acidity and high metallic and sulphate content, that originates in mining areas containing sulphide ores. The process of AMD generation starts with the direct oxidation of sulphides to sulphates, accompanied with an increment of acidity, and followed by indirect oxidation with the ferric ion. As a result of these reactions, several elements (e.g. Fe, Cu, Al, Mn, As, Pb, Co, Zn) are stored as soluble compounds. The lixiviates coming from the mines and waste rock heaps produce aqueous discharges loaded in AMD that represents a potential source of contamination for the underground and superficial water courses that receive them [3]. This is the case of the Odiel River, in the province of Huelva (SW Spain), which crosses in its upper course the Iberian Pyrite Belt, one of the most important metallogenic regions in the world [4,5]. The arsenic found in the waters of the Odiel River comes from the oxidation of As rich

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pyrites (with up to a 0.4% of As), together with minor amounts of arsenopyrite and other As containing minerals [6]. The AMD originated by mining activity represents a daily transport of this river of at least 500 t of contaminants (sulphates and metals) along its course to its estuary [7].

The preservation of the distribution of As species in water samples is a difficult task, as the sample matrix can produce quick changes in the oxidation states. The literature reports the capability of the organic matter both to oxidise As(III) to As(V) and to reduce As(V) to As(III), even after sample filtration [8,9]. The most common approaches to preserve the As species include the addition of acidic solutions of HCl, HNO_3, H_2SO_4, H_3PO_4, nitriloacetic acid, ascorbic acid [10–15] and complexing agents (EDTA) [16] in combination with temperature control (room temperature, refrigerated or freeze). However, the results found by many authors are somehow contradictory [17]. Sometimes no significant changes are found after several days of storage, whereas in other cases oxidation or reduction after few hours. A recent article reviews the published literature referred to As(III) and As(V) determinations in water samples and estates the many discrepancies, confusion and ambiguity of the results found by several authors concerning the stability of the As species using different preservation techniques [18]. Its main recommendations are that a field collection should filter out microorganisms, a reagent should be added to prevent dissolved Fe and Mn oxidation and precipitation, and to isolate the sample from solar radiation to avoid photochemical reactions.

Of the several preservation studies of water samples in the literature for As speciation, few of them consider AMD samples [19,20], due to the complexity of the sample matrix. After filtration, the AMD sample is collected in opaque bottles and an EDTA solution is added to sequester the Fe, Mn, and Al cations. The arsenic species are determined by HPLC-ICP-MS. They propose also a field speciation method based on solid-phase extraction cartridges, in which the sample is initially filtered and treated with EDTA and then passed through a cartridge containing a strong anion exchange resin in the acetate form, where arsenate is retained, whereas arsenite (an uncharged species) elutes. Arsenate is eluted afterwards from the cartridge with a nitric acid solution. However, the existing preservation methods may not be suitable for all kind of AMD samples, as the metallic matrix may be very different. This is the case of AMD with extremely high metallic load, as it happens in the ones considered in the present work. This makes necessary to adapt or develop new preservation methods.

The purpose of our work is to study and develop a new preservation method for arsenic speciation in AMD samples, based on the acidification of the sample and in situ cleanup using a cationic exchange resin. We have investigated the influence of several parameters on the arsenic species stability along a period of time from few hours until 1 week, such as the material of the sample containers (glass, polyethylene), the temperature (ambient, refrigerated and frozen), and the addition of reagents that have been commonly employed in other well established methods (AEDT, acids). The development of a suitable preservation method for AMD sample is the first stage of a project to evaluated the contamination of arsenic species in the Odiel River, caused by the lixiviates coming from the mines lixiviates and the ubiquitous waste rock heaps that are present in its basin.

2. Experimental

2.1. Reagents

Standard solutions of 1000 mg L^{-1} (as As) were prepared based on arsenic trioxide (Panreac, Barcelona, Spain) and sodium arsenate (Merck, Darmstadt, Germany). Calibration solutions of 5, 10, 25 and 50 μg L^{-1} containing both As species were daily prepared. The different reagents employed in the HPLC-HG-AFS determinations (HCl, $NaBH_4$, NaOH, KH_2PO_4, K_2HPO_4), or in sample preservation (EDTA di sodium salt, HCl, AcH) were of analytical grade (Merck, Darmstadt, Germany). The different solutions were prepared with Milli-Q (18.2 MΩ) water. Cationic exchange resin Amberlite 120-IR Na (Rohm and Hass SAS, Chauny, France) was employed for sample cleanup.

2.2. Instrumentation

Electrical conductivity, pH were measured in the field and in the lab, using a portable MX 300 measurer (Mettler Toledo, USA). The redox potential was also measured in the field using HANNA measurer with Pt and Ag/AgCl electrodes (Crison, Barcelona, Spain). All samples were filtered using a 0.2 μm pore size cellulose acetate membrane (Albet, Barcelona, Spain).

Arsenic speciation of the AMD samples was achieved by coupled HPLC-HG-AFS. This instrumental coupling allows detection limits below the μg L^{-1} level. To summarize the analytical process, 200 μL of sample are injected into a 25 cm long Hamilton PRP-X100 strong anionic exchange column (Hamilton, Reno, USA) using a 25 mmol L^{-1} phosphate buffer (pH 5.8) as the mobile phase, at a flow rate of 1.1 mL min^{-1} (Jasco PU 1580 HPLC pump). The order of elution is As(III) and As(V). Each chromatographic run lasts 12 min. HCl 1 mol L^{-1} and 1% (w/v) $NaBH_4$ solutions are continuously added at the outlet of the chromatographic column by means of a peristaltic pump. The volatile arsines generated are transported with the aid of an argon flow to a glass gas–liquid separator. The gaseous phase is dried from the water moisture using a hygroscopic membrane before entering the hydrogen-air flame of the AFS detector (Excalibur 10.33, PS Analytical, Orpington, Kent, UK). This instrumental set up for arsenic speciation has been previously described elsewhere [21].

2.3. Sample point location

The study area is located near the village “Almonaster la Real” (province of Huelva, SW Spain), within the so-called Iberian Pyrite Belt, a volcanogenic massive sulphide deposit in exploitation since pre-historical times [4]. For this study, samples of AMD derived from the “Cueva de la Mora” mine were used, where until 1960s pyrite and chalcopyrite ores were the main mined products together with minor amounts of arsenopyrite, pirrotite, sphalerite and galena as accessories. The AMD

samples were taken from an effluent that drains out of one of several shafts connected to underground galleries of the old mine.

2.4. Sample collection and preservation procedures

Four sampling campaigns were undertaken on days 19th July, 24th August, 25th October and 1st December of 2004. In all of them pH, electrical conductivity, and redox potential were in situ measured. In each campaign, 250 mL portions of the effluent of "Cueva de la Mora" were filtered in the field using 0.2 μm pore size filters, and divided into several smaller samples for the different preservation methods tested. For each preservation method, duplicate samples were always considered. In the lab, the arsenic speciation analysis of the samples was undertaken by HPLC-HG-AFS. In order to study the samples stability between collection and analysis, the samples were analysed at time intervals of 3 h during the first 12 h, then hereafter at 24, 48 h and 1 week.

The preservation of the arsenic species was studied as a function of the recipient used, the preservation temperature, and the chemical reagents added. Initially, 50 mL opaque glass and polyethylene bottles were used. After collection, the temperature of the samples was maintained either at −18 °C (frozen with dry ice), refrigerated at approximately 4 °C (in an isothermic box or in fridge), or maintained at ambient temperature (20 °C in the lab). When samples were preserved with EDTA, the standard procedure was to add 2 mL of a 0.25 mol L^{-1} EDTA solution to 20 mL of filtered sample, although in some experiences 0.12 mol L^{-1} EDTA was also employed. Two acidification procedures for preservation were tested: 60 μL of 6 mol L^{-1} HCl or 200 μL of 8.7 mol L^{-1} AcH were added to 20 mL of filtered sample. Ion exchange cleanup of the samples was also tried to remove metallic cations in the samples: 30 mL aliquots of filtered sample (not acidified or acidified with HCl 6 mol L^{-1}) were eluted at a flow rate of ca. 2 mL min^{-1} through 10 g a cationic exchange resin placed in a glass column (25 cm height, 1.5 cm i.d.). After elution of the first 10 mL of sample, the remaining 20 mL were collected.

3. Results and discussion

3.1. Chemical characterization of the samples

The pH, redox potential and conductivity of the samples of the four sampling campaigns were measured in the field and also in the lab. The pH of all the samples was very similar, not depending on the sampling campaign, the mean pH being 3.30 ± 0.04. The mean redox potential of the samples was 390 ± 22 mV. The low pH at moderately oxidizing conditions are an indication that both As(III) and As(V) may be present in the samples, according to Eh/pH diagrams. The high dissolved metallic content of the samples is related to the high conductivity measured, 4.0 ± 0.8 mS cm^{-1}. Concentration up to 455 μg L^{-1} of As and 307 mg L^{-1} Fe in this effluent have been determined [5]. Concentration of other metals has been also measured: 99.5 mg L^{-1} Al, 4.5 mg L^{-1} Cu, 24 mg L^{-1} Mn, 97.5 mg L^{-1} SiO_2, 399.6 mg L^{-1} Zn, 319 mg L^{-1} Mg, 312 mg L^{-1} Ca, 0.17 mg L^{-1} Pb, 0.9 mg L^{-1} Ni, 1.1 mg L^{-1} Co, 0.3 mg L^{-1} Cd and 4117 mg L^{-1} of sulphate (A.M. Sarmiento, unpublished work).

3.2. Type of container, EDTA addition and temperature control for preservation of as species

The oxidation of arsenite to arsenate by the Fe(III) cation together with oxygen is the main redox reaction that has to be avoided in order to preserve the AMD samples. Also, the precipitation of ferric compounds stimulates the co-adsorption of arsenic species [15]. EDTA was chosen as quelating agent to minimize the Fe interference, as it has been previously employed in the preservation of AMD samples [19]. EDTA has the capability to form 1:1 complexes with most metals, depending on the pH, regardeless of their charge.

In the first sampling campaign, a first set of samples were filtered, collected either in opaque glass bottles or polyethylene bottles, and EDTA added to all of them. The amount of EDTA added (2 mL of 0.25 M EDTA to 20 mL of sample) was chosen to make sure that EDTA was in excess, considering the high metallic content of the current samples. This amount is double that the quantity considered by some authors with other AMD samples [19]. This first set of samples correspond to the established procedures describes in the literature for sample preservation [19]. Some samples were stored at ambient temperature, others were refrigerated or frozen.

A second set of samples from the same sampling point was also filtered, but instead of adding EDTA, the metallic content was diminished in situ by cleanup using glass columns filled with a cationic exchange resin, collected in opaque bottles and refrigerated. This second set of samples corresponds to the possible new method for sample preservation that we investigated. A scheme of the different procedures is depicted in Fig. 1.

The results corresponding to arsenic speciation analysis are shown in Table 1. The mean concentrations of As(III) and As(V) of the samples of the first set was estimated in 38 ± 3 and 238 ± 6 μg L^{-1}, respectively. These means were determined considering the first measurements realised 3 h after collection (samples 1–4), as the results among them did not differ significantly. No methylated species were found. It can be observed for the samples of the first set, that if we compare two of them under the same conditions of temperature that the material of the container does not affect the results. In this case, the opaque glass bottles were preferred to the polyethylene ones for further sampling campaigns, as the literature recommends to avoid light that may cause undesirable photochemical reactions [18].

The results corresponding to preservation experiences with EDTA (samples of set 1 in Table 1) at ambient temperature (samples 1 and 2) were not satisfactory, mainly for As(V), whose concentration increased continuously during the first 12 h and the next 2 days. The use of refrigeration in combination with EDTA (samples 3 and 4) did not improve the preservation. The increment in the As(V) in these samples may be due to a desorption of this specie from the Fe oxyhydroxides colloids present in the filtered samples, onto which arsenate easily bounds [22]. The addition of EDTA 0.25 mol L^{-1} to the samples produced

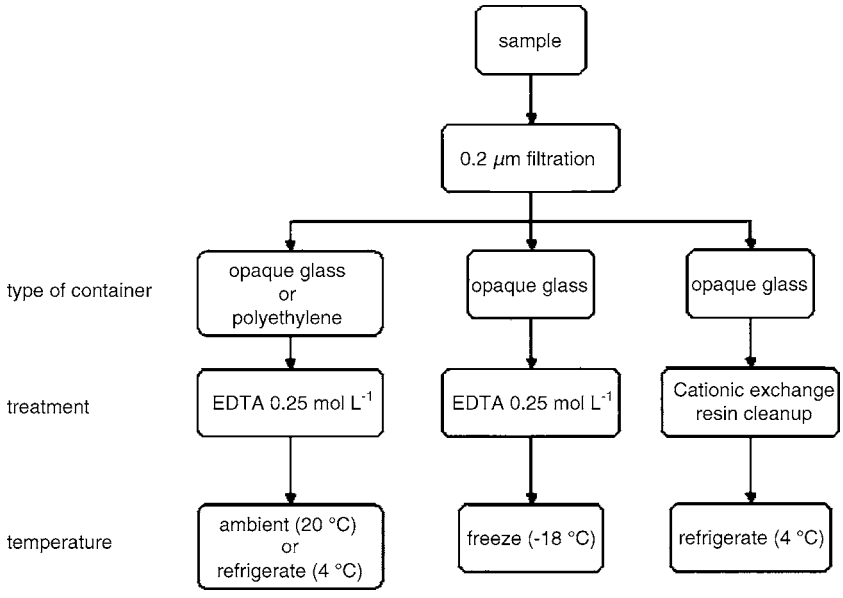

Fig. 1. Schematic diagram of the preserving methods for the first and second sampling campaigns.

an initial diminution of ca. 0.7 units of the pH of samples (from pH 3.30 ± 0.04 to 2.52 ± 0.22), which increases afterwards ca. 0.4 pH units along the 1 week time of the analysis, which could enhances the desorption process. The desorption of As(V) from Fe oxyhydroxides when pH increases has been previously reported [23]. If samples are treated with EDTA and frozen (sample 5), it can be observed that since the first analysis the results are not accurate, specially for As(V), as the concentrations found for the frozen sample ranges 284–300 $\mu g\,L^{-1}$ during the first 3–48 h period, significantly higher compared to the mean initial 238 $\mu g\,L^{-1}$ for As(V). Changes of distribution of As in frozen samples after defrosting have been reported in

Table 1
Preservation conditions for speciation of arsenic in AMD samples: type of container (opaque glass or polyethylene), EDTA addition, temperature control (ambient temperature, refrigerated or frozen) and resin cleanup

	Time after sampling						
	3 h	6 h	9 h	12 h	24 h	48 h	1 week
Set 1 (EDTA addition)							
Sample 1: (EDTA 0.25 mol L^{-1}, opaque glass, ambient temperature)							
As(III)	38.0 ± 0.5	37.7 ± 0.7	35.3 ± 0.8	35.2 ± 0.7	31.1 ± 0.4	22.2 ± 0.3	25.3 ± 0.5
As(V)	242.1 ± 13.8	252.4 ± 16.6	280.4 ± 17.2	287.9 ± 15.9	322.3 ± 5.3	318.3 ± 18.8	242.1 ± 12.5
Sample 2: (EDTA 0.25 mol L^{-1}, polyethylene, ambient temperature)							
As(III)	37.9 ± 0.2	42.4 ± 0.6	42.0 ± 0.5	39.4 ± 0.4	47.3 ± 0.4	43.9 ± 0.7	41.8 ± 0.4
As(V)	242.2 ± 1.7	260.9 ± 1.9	282.5 ± 1.9	291.2 ± 1.8	332.0 ± 1.6	333.0 ± 2.1	307.3 ± 1.6
Sample 3: (EDTA 0.25 mol L^{-1}, opaque glass, refrigerated)							
As(III)	35.4 ± 0.2	38.6 ± 0.1	38.1 ± 0.3	38.7 ± 0.2	44.6 ± 0.3	43.9 ± 0.0	34.2 ± 0.2
As(V)	240.6 ± 1.1	253.0 ± 0.4	265.2 ± 1.6	275.3 ± 1.0	318.7 ± 2.4	294.4 ± 0.1	311.9 ± 0.9
Sample 4: (EDTA 0.25 mol L^{-1}, polyethylene, ambient temperature)							
As(III)	42.7 ± 0.5	38.9 ± 0.6	39.8 ± 0.6	40.0 ± 0.6	47.9 ± 0.4	47.2 ± 0.7	43.2 ± 0.5
As(V)	228.4 ± 15.6	237.8 ± 16.0	257.0 ± 15.9	237.1 ± 15.8	291.9 ± 13.3	289.7 ± 16.4	267.7 ± 13.3
Sample 5: (EDTA 0.25 mol L^{-1}, opaque glass, frozen)							
As(III)	40.7 ± 2.0	n.m[a]	n.m.	n.m.	45.6 ± 3.9	45.7 ± 0.0	41.3 ± 2.0
As(V)	284.2 ± 7.2	n.m.	n.m.	n.m.	299.6 ± 14.2	294.4 ± 0.1	277.2 ± 7.2
Set 2 (resin cleanup)							
Sample 6: (No EDTA added, opaque glass, refrigerated)							
As(III)	44.6 ± 0.8	41.1 ± 1.2	40.6 ± 0.2	44.4 ± 0.7	49.2 ± 1.3	45.9 ± 0.4	19.6 ± 0.7
As(V)	66.2 ± 3.2	82.8 ± 1.0	82.4 ± 4.6	81.3 ± 2.9	109.5 ± 3.9	104.6 ± 3.4	47.6 ± 2.7
Sample 7: (No EDTA added, polyethylene, refrigerated)							
As(III)	42.1 ± 0.3	42.7 ± 0.7	43.2 ± 0.4	45.0 ± 0.5	49.4 ± 0.4	48.2 ± 0.2	34.3 ± 0.4
As(V)	71.6 ± 0.3	94.0 ± 0.4	97.6 ± 0.3	99.4 ± 0.3	125.3 ± 0.3	115.95 ± 0.4	109.6 ± 0.3

First sampling campaign 19th July 2004. Result expressed as μg As $L^{-1} \pm$ standard deviation ($n = 2$). The mean initial concentrations are $38 \pm 3\,\mu g\,L^{-1}$ for As(III) and $238 \pm 6\,\mu g\,L^{-1}$ for As(V).

[a] Not measured.

other arsenic speciation studies, assuming that the oxidation by the Fe ions is favoured by the freezing/defrosting process [15]. We can conclude from the results with EDTA, that the samples seem not to be stable for more than a few hours. These results are in apparent contradiction with other studies in which the AMD samples treated with AMD were stable for 3 months [19], probably due to differences in the matrix of the samples. This is may be explained considering that the AMD samples of our study are characterized by high concentration of Fe (hundreds of $mg\,L^{-1}$) present not only as Fe (II) but also as Fe(III), thus representing a serious source for alteration of the distribution of the arsenic species due to redox reaction and co-adsoprtion on the Fe oxyhydroxides. This situation is not found in AMD samples from other mining areas (like Carnoulès in France), where the Fe content may also be high (up to 34.4 $mmol\,L^{-1}$) but all of it present as Fe(II), and samples are analysed within 24 h without any chemical treatment [24].

3.3. Resin cleanup for preservation of as species

A first approach to eliminated in situ the Fe interference based on the use of a cationic exchange resin (Amberlite IR-120) was tried. The resin would retain the metallic cations species, but not the arsenic ones (arsenate is negatively charged, whereas arsenate is neutral at the samples pH of 3.3). This resin cleanup has been previously reported for the reduction of the metallic content in water samples [25]. The removal of the cations in the samples was checked, determining the concentration of several heavy metals (Fe, Mn, Cu, Zn, Al, Pb, Cd, Co, Mg, Ca and Ni) by ICP-AES (inductively coupled plasma-atomic emission-spectroscopy). The results obtained before and after the sample cleanup indicated a removal of 98–100% of the metals in solution.

It can be seen in the Table 1 (samples 6 and 7 of set 2) that the As(III) concentration was similar to the values found in the first set of samples, whereas As(V) was partially retained in the column, as the As(V) concentration found since the first analysis were very low (72 and 66 $\mu g\,L^{-1}$ with glass and plastic bottles) in comparison to the estimated mean of 238 $\mu g\,L^{-1}$. As it has been mentioned before, As(V) bounds easily onto the Fe oxyhydroxides colloids, which have a positively charged surface at low pH, and may be either partially retained in the cationic resin or co-adsorbed onto a Fe precipitate, as pH increases from 3.30 ± 0.04 to 4.40 ± 0.30 when samples are eluted through the resin.

We can conclude from the results obtained in this first sampling campaign that the preservation methods described in the literature, based mainly on the use of EDTA, maintain the integrity of the samples only a few hours (no more than 3 h), a time that usually is too short between sampling and analysis. This results were confirmed in a second sampling campaign was performed on 24th August 2004. Samples were collected, preserved and analysed in the same way as in the previous campaign. The bulk As content of the samples had diminished; the mean As concentration of As(III) concentration was of the same order as before, but a little lower, (mean 26 $\mu g\,L^{-1}$), whereas the As(V) concentration had lowered to a mean value of 53 $\mu g\,L^{-1}$. The tendency of the results were identical to those ones found in the sampling campaign and therefore are not shown; the EDTA addition was not able to preserve the samples, either at ambient temperature of refrigerated, for more than a few hours, and As(V) was partially retained if the cationic resin was employed for sample clean up.

3.4. Influence of acidification on previous preservation methods

New approaches to sample preservation were performed, based on the acidification of the samples, always in combination with resin cleanup or EDTA addition. Acidification is widely use for water samples preservation, as is the case of AMD, because it avoids the precipitation of Fe oxyhidroxides. HCl or HNO_3 are the most common acid employed for water sample preservation. Of the two, HCl was chosen for acidification, as it is used in official methods for water samples preservation in arsenic speciation determination [14], whereas HNO_3 was discarded, as it has been reported to alter the arsenic species distribution [12]. AcH was also tried, as it has been described to prevent iron–arsenic coprecipitation [26]. However, it should be bear in mind that acidification can have a negative effect if samples are to be preserved with EDTA, as the complexation efficiency of EDTA with metals depends on the pH, as the conditional formation constant of the complex of EDTA with cationic metals diminishes when the pH increases. The concentration of the EDTA solution was also investigated. EDTA 0.12, 0.25 and 0.50 $mol\,L^{-1}$ solutions were tried, although the last one was discarded due to solubility problems. Preservation just based on sample acidification was probed to be ineffective for practical reasons, as the HPLC column was irreversible affected by huge Fe precipitates that appears when the sample is injected onto the chromatograph an gets mixed with the phosphate buffer (pH 5.8).

During the third sampling campaign (25th October 2004), all samples were taken in opaque glass containers and refrigerated. A sample not acidified and with added 0.25 $mol\,L^{-1}$ EDTA, as in the first campaign, was also taken for comparison. A scheme of the different procedures is depicted in Fig. 2. Again, two sets of samples were collected: the first set of samples corresponds to those with EDTA added at different concentrations and HCl or AcH for acidification. The second set of samples corresponds to those with resin cleanup, acidification with HCl and no EDTA addition.

The results corresponding to the arsenic speciation analysis are summarized in Table 2. The mean As(III) measured (157 $\mu g\,L^{-1}$) was higher than the As(V), 135 $\mu g\,L^{-1}$. As expected, sample 1 (with EDTA 0.25 $mol\,L^{-1}$ added and no acidification), confirmed the same trend found in the two previous campaigns; a diminution of the As(III) concentration, and an increase of As(V) with time. Also, the acidification (either with HCl or AcH), in combination with EDTA (either at 0.12 or 0.25 $mol\,L^{-1}$), confirmed the expected results (samples 2–4 of set 1 in Table 2): the capability of EDTA to preserve the sample was diminished. From the results of this set of samples, we can conclude that a high EDTA concentration helps to stabilize the arsenic species in the AMD samples, but only for a few hours,

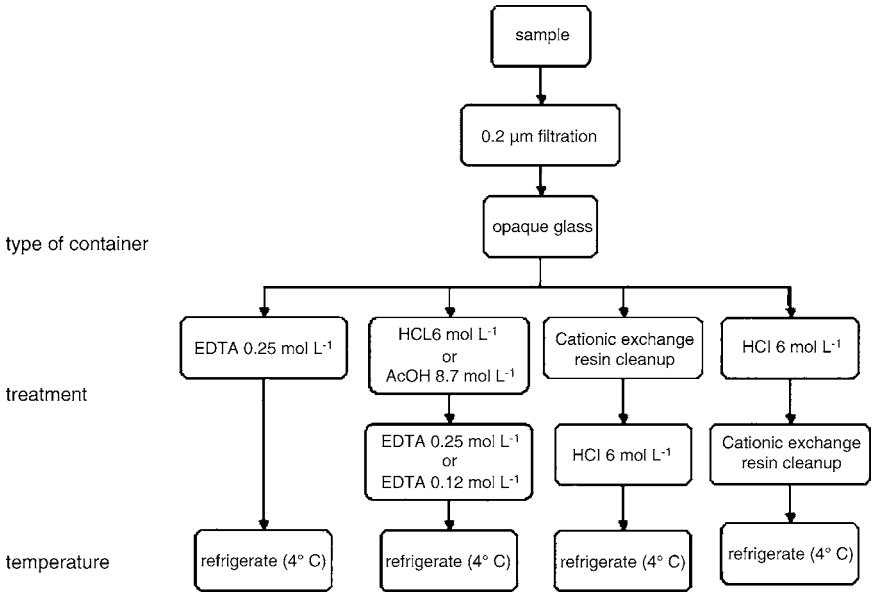

Fig. 2. Schematic diagram of the preserving methods for the third and fourth sampling campaigns. In bold type, the conditions corresponding to the new proposed method.

and that acidification of the samples may inhibit the precipitation of Fe, but its combination with EDTA reduces the stability of the samples.

Other experiences were tried in order to improve the cleanup procedure with the cationic resin, which correspond to the results of the second set of samples in Table 2. As indicated in previous experiences, As(V) was partially retained in the resin employed for sample cleanup, probably due to the adsorption onto the Fe-rich colloids which bound to the resin. Sample 5 (in situ resin cleanup and then acidification with HCl) confirms this hypothesis. The results, as it happened in previous experiences, produced a drastic reduction of the As(V) from 135 to 20.5 $\mu g\,L^{-1}$ only 3 h after collection. In order to avoid the adsorption of As(V), sample 6 was first acidified with HCl and then eluted through the resin. As it can be seen in Table 2, the results were satisfactory both for As(V) and As(III) for at least 48 h. After 1 week, a small loss in the As(III) was found. There was also a small increase of the pH of the sample (from pH 3.30 to 3.70), when the sample was eluted through the column, with no further changes in the period of study.

Table 2
Preservation conditions for speciation of arsenic in AMD samples: influence of acidification on previous preservation methods

	Time after sampling						
	3 h	6 h	9 h	12 h	24 h	48 h	1 week
Set 1 (EDTA addition)							
Sample 1: (EDTA 0.25 mol L^{-1})							
As(III)	164.0 ± 0.7	147.8 ± 1.1	124.4 ± 1.1	118.0 ± 0.1	116.0 ± 0.1	83.1 ± 0.4	83.7 ± 0.7
As(V)	121.8 ± 1.0	156.0 ± 2.4	158.0 ± 4.5	170.4 ± 0.5	207.1 ± 13.8	160.4 ± 12.8	213.9 ± 0.8
Sample 2 (EDTA 0.25 mol L^{-1} and HCl 6 mol L^{-1})							
As(III)	153.5 ± 5.4	115.8 ± 13.1	83.4 ± 14.9	70.2 ± 0.3	60.6 ± 0.4	37.6 ± 0.5	7.5 ± 0.1
As(V)	141.7 ± 7.7	196.7 ± 19.1	206.8 ± 10.9	208.2 ± 1.3	222.8 ± 1.0	209.7 ± 3.1	293.2 ± 8.3
Sample 3:(EDTA 0.12 mol L^{-1} and HCl 6 mol L^{-1})							
As(III)	96.2 ± 0.1	40.4 ± 2.5	18.6 ± 1.0	10.8 ± 1.0	0	0	0
As(V)	236.0 ± 5.2	266.1 ± 14.9	268.9 ± 7.0	261.2 ± 5.2	297.2 ± 5.8	227.4 ± 1.6	290.6 ± 3.1
Sample 4: (EDTA 0.25 mol L^{-1} and AcOH 8.7 mol L^{-1})							
As(III)	157.8 ± 2.8	135.6 ± 6.6	117.0 ± 3.1	110.8 ± 1.1	118.2 ± 2.4	81.0 ± 1.2	82.4 ± 7.3
As(V)	142.6 ± 5.7	170.6 ± 7.6	153.8 ± 7.1	154.3 ± 4.0	170.8 ± 8.5	146.4 ± 1.3	198.7 ± 13.4
Set 2 (resin cleanup)							
Sample 5: (HCl 6 mol L^{-1} added after resin cleanup)							
As(III)	160.6 ± 1.6	153.8 ± 3.9	147.3 ± 2.1	140.9 ± 0.7	150.5 ± 0.8	104.9 ± 1.0	135.5 ± 0.3
As(V)	20.2 ± 3.6	24.0 ± 1.1	23.5 ± 0.8	28.3 ± 2.2	29.2 ± 0.8	22.6 ± 2.8	32.6 ± 6.7
Sample 6: (HCl 6 mol L^{-1} added before resin cleanup)							
As(III)	151.7 ± 3.0	149.2 ± 1.8	144.9 ± 2.2	137.6 ± 3.6	140.3 ± 7.8	140.0 ± 0.7	136.1 ± 2.8
As(V)	132.4 ± 8.7	135.0 ± 10.2	132.6 ± 8.9	130.2 ± 9.6	133.0 ± 7.3	130.5 ± 0.7	129.4 ± 8.6

All samples were stored in opaque glass containers and refrigerated (4 °C). Third sampling campaign 25th October 2004. Result expressed as μg As L^{-1} ± standard deviation ($n = 2$). The mean initial concentrations are 157 ± 5 $\mu g\,L^{-1}$ for As(III) and 135 ± 10 $\mu g\,L^{-1}$ for As(V).

Table 3
Preservation conditions for speciation of arsenic in AMD samples: comparison of EDTA addition and the new preservation method proposed

	Time after sampling						
	3 h	6 h	9 h	12 h	24 h	48 h	1 week
Set 1 (EDTA addition)							
Sample 1: (EDTA 0.25 mol L^{-1})							
As(III)	39.6 ± 0.8	44.2 ± 0.0	35.1 ± 0.4	46.9 ± 0.2	42.4 ± 3.5	40.1 ± 2.1	36.8 ± 0.8
As(V)	33.6 ± 3.4	70.1 ± 3.6	66.0 ± 3.5	60.4 ± 6.0	33.0 ± 1.1	30.2 ± 2.1	39.5 ± 0.8
Set 2 (resin cleanup)							
Sample 2: (HCl 6 mol L^{-1} added before resin cleanup)							
As(III)	37.9 ± 0.4	36.9 ± 1.0	38.6 ± 0.7	37.8 ± 0.5	35.9 ± 0.7	36.4 ± 3.5	32.2 ± 1.5
As(V)	31.1 ± 0.4	31.8 ± 1.9	30.5 ± 1.2	30.6 ± 0.2	30.8 ± 0.5	32.8 ± 2.0	35.2 ± 3.3

All samples were stored in opaque glass containers and refrigerated (4 °C). Fourth sampling campaign 1st December 2004. Result expressed as μg As L^{-1} ±standard deviation ($n = 2$). The mean initial concentrations are 38 ± 2 μg L^{-1} for As(III) and 32 ± 2 μg L^{-1} for As(V).

The stability of the samples during the 3 h elapsed between sample cleanup and analysis was also considered. The extrapolation to time 0 h of the results in Table 2 (set 2, sample 6) and Table 3 (set 2, sample 2) indicate that no changes should occurs, as the representation of the results for As(III) and As(V) indicated horizontal lines. This was confirm with simulated AMD samples prepared in the lab, containing a similar metallic matrix as the real samples and with added arsenic species. The immediate analysis of the simulated samples after resin cleanup did not show any change in the distribution of the arsenic species.

The good results obtained with this method were confirmed in a fourth campaign (1st December 2004, Table 3). In this case, only two preservation methods we employed; the more classical reported in the literature for AMD, consisting of the addition of EDTA without acidification, and the new one here proposed, based on acidification with HCl and cationic resin cleanup. The results indicated similar As(III) and As(V) concentrations, mean 38 and 32 μg L^{-1}, respectively. The tendency of results is clear: similar results with both methods were obtained only during the first analysis (3 h after sample collection). After that time, the preservation method based on the use of EDTA showed significant changes, specially for As(V), which increased its concentration two-fold 9 h after sampling, decreasing afterwards. On the other hand, the new method proposed, corresponding to sample 2, indicated no significant changes for As(III) and As(V) for at least 48 h, as the As(III) dimished ca. 15% after 1 week. This results confirm the capability of the new preservation method that we propose to stabilize the arsenic species in AMD samples for an extended period of time of at least 48 h, usually enough for the time elapsed between sampling and posterior analysis.

4. Conclusions

The preservation of AMD samples for arsenic speciation studies represents a difficult task. Common approaches based on the use of EDTA and refrigeration allows only preserving AMD samples for a few hours. The new preservation method proposed based on the acidification of the sample with HCl, refrigeration and in situ sample cleanup with cation exchange resin removes the interferences caused by metallic cations (e.g. Fe). With this method, As species were stable for al least 48 h. The development and optimisation of a suitable preservation method is a first step of the analytical procedure to evaluate input of arsenic species caused by AMD lixiviates into the Odiel River basin.

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