Talanta 104 (2013) 187–192

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Hydride generation using a metallic atomizer after microwave-assisted extraction for inorganic arsenic speciation in biological samples

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ARTICLE INFO

Article history:
Received 3 September 2012
Received in revised form
5 November 2012
Accepted 6 November 2012
Available online 16 November 2012

Keywords:
Speciation analysis
Mild conditions
Hydride generation
Sample preparation
MAE

ABSTRACT

The present speciation method reports the determination of inorganic arsenic forms, using metallic furnace hydride generation atomic absorption spectrometry. The inorganic As speciation is carried out using mild conditions for hydride formation, such as slightly acid pH media (4.50) and low tetrahydridoborate(1−) concentration (0.1% (w/v)). Limits of detection and quantification of 2.0 and 6.6 $\mu g\,L^{-1}$ of iAs(III) are obtained using optimized conditions. Additionally, microwave-assisted extraction using water as solvent is carried out to provide the appropriate environment for As species extraction as well as impeding interconversion between species. With these analytical strategies, As was accurately determined (at 99.9% confidence level) in water and plankton samples.

1. Introduction

There is no doubt that atomic absorption spectrometric determination of As is currently carried out using hydride generation techniques with quartz tube atomizers (QTA) [1–4], to obtain high detectivity and selectivity, compared to the flame mode [5,6].

Figueiredo et al. [7] recently proposed an alternative to QTA, based on hydride generation metal furnace atomic absorption spectrometry (HG-MF-AAS), focusing on Sb determination in river and lake sediments, and in pharmaceuticals. After this, Klassen et al. [8] successfully determined Bi and Se using a metallic atomizer in medical and urine samples, as well as total As in animal and sediment samples [9]. For all these methods, besides good figures of merit, the authors highlighted the low cost for its implementation (*ca.* US$ 7/tube), good tube life-time (> 2000 h), long-term stability and robustness.

Since the surfaces of these furnaces are reactive, which depends on the alloy used and the flame conditions, new environments can be obtained, making possible different reactions on these surfaces, and allowing efficient hydride decomposition [7–9]. Taking into account the Inconel 600® alloy for As determination [9], the Ni present in the surface of this atomizer can react with As, forming $Ni_3(AsO_4)_2$ ($\Delta^{\circ}G_f = -1581.73\ \mathrm{kJ\,mol^{-1}}$), which decreases the analytical signal. However, after the injection of a high concentrated standard of As (40 $\mathrm{mg\,L^{-1}}$), Ni was removed from the furnace surface by forming a volatile species, increasing the analytical signal [9]. The same behavior was also noted for Sb determination using the same alloy [7].

Besides these good characteristics of the metallic atomizer for hydride generation purposes, when focusing on speciation analyses, it is important to pay attention to sample preparation for avoiding changes in the species distribution of the analytes. Thus, microwave-assisted extraction using water as solvent may provide an alternative method for sample preparation before speciation analyses [10,11].

Although metallic atomizers present good characteristics for analytical purposes, their use for speciation analysis has not yet been reported in the literature. Due to the importance of As speciation [12–14], the novelty of hydride generation and the use of metallic tube atomizer for speciation, a method for inorganic As (iAs) speciation is proposed herein.

Another purpose of this work is to improve sample preparation using microwave-assisted extraction (MAE) in closed vessels with water as solvent for obtaining mild extraction conditions in order to preserve the As species in solution, as well as to use mild conditions (slightly acid pH condition and low tetrahydridoborate(1−) concentration) in the hydride generation for avoiding reduction of iAs(V).

2. Experimental

2.1. Instruments and apparatus

A Perkin-Elmer AAnalyst 300 flame atomic absorption spectrometer (Shelton, USA) equipped with deuterium lamp background

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http://dx.doi.org/10.1016/j.talanta.2012.11.009

correction and PE-AA WinLab software was used for trivalent inorganic arsenic (iAs(III)) and total inorganic arsenic (iAs_t) determinations. The concentration of pentavelent inorganic arsenic (iAs(V)) was estimated as the difference between iAs_t and iAs(III).

Electrodeless discharge lamps (EDL) were used as primary radiation sources, and operating conditions were those recommended by the manufacturer ($\lambda = 193.7$ nm and 0.7 nm slit, and 300 mA). All measurements were based on integrated absorbance.

The on-line hydride generation system consisted of an Ismatec peristaltic pump (IPC-12, Glattzbrugg, Switzerland), and a polymethacrylate three-piece injector–commuter device designed and built in our laboratory. Polyethylene tubes (0.7 mm i.d.) were used as transmission lines, and Tygon® tubes were used for propelling the solutions. An Inconel 600® metallic furnace (Ni 72.0% as minimum, Cr 14.0–17.0%, Fe 6.0–10.0%—Camacam, São Paulo, Brazil) was used over an air:acetylene flame as atomizer, as described elsewhere [7–9].

A Perkin-Elmer Model AAnalyst 600 electrothermal atomic absorption spectrometer (GF AAS, Shelton, USA) with a transversely heated graphite tube atomizer (THGA), a Zeeman effect background corrector and an AS-800 autosampler was used for total arsenic and accuracy determination. All measurements were based on integrated absorbance using an electrodeless discharge lamp (Perkin-Elmer, Shelton, USA) for As (all conditions as already described above or as recommended by the manufacturer).

A Digimed DM20 potentiometer (São Paulo, Brazil) coupled to a Pt/Ag/AgCl, with saturated KCl gel as internal electrolyte, as well as a pH electrode was used for potential and pH evaluations.

For the microwave-assisted extraction procedure, a Provecto Analítica microwave oven (Jundiaí, Brazil) was used.

2.2. Reagents and solutions

All solutions were prepared with deionized water (18 MΩ cm) obtained from a Milli-Q water purification system (Millipore, USA). A solution of Na_3AsO_3 (Riedel-de Haën, Germany), 0.05 mol L^{-1} in water, and another of $Na_2HAsO_4 \cdot 7\,H_2O$ (Aldrich, USA) dissolved in 2% (v/v) HCl (Merck, Germany), both at 1000 mg L^{-1} of As, were used as sources of iAs(III) and iAs(V), respectively. A solution of $NaBH_4$ (THB) (Aldrich, USA) preserved in 0.05% (w/v) NaOH (Sigma Aldrich, Sweden) was used as hydride source. Anhydrous citric acid (Synth, Brazil) was used for preparing the citrate buffer at pH 4.50 (adjusted with NaOH). The pre-reducing agents were KI (Nuclear, Brazil) plus ascorbic acid (Merck, Brazil), $NH_2OH \cdot HCl$ (Merck, Germany), $Na_2S_2O_3$ (Fluka, USA), L-cysteine (Aldrich, USA) and thiourea (Synth, Brazil). The reagents $K_4Fe(CN)_6 \cdot 3\,H_2O$ (Aldrich, USA) and $K_3Fe(CN)_6$ (J.T. Baker, Mexico) were used to prepare the standard of 228 mV for the Pt/Ag/AgCl electrode at 5×10^{-4} mol L^{-1} for each salt, dissolved in a 0.1 mol L^{-1} phosphate buffer (pH 7). For preparing this buffer, an equimolar solution of KH_2PO_4 and K_2HPO_4 (J.T. Baker, Mexico) was used, and the pH was adjusted to 7 with 1 mol L^{-1} NaOH. Commercial buffer solutions of phosphate (at pH 7) and phthalate (at pH 4) from Quemis (Joinville, Brazil) were used for the calibration of the pH electrode. The reagents $Mg(NO_3)_2 \cdot 6H_2O$ (Mallinckrodt, USA) and $Pd(NO_3)_2 \cdot H_2O$ (Aldrich, USA) were used as matrix modifier for GF AAS analyses, following the manufacturer's recommendations. Both NIST 1643e (USA), trace elements in water, and BCR414 (Belgium), plankton, were used as certified materials for accuracy purposes.

2.3. System optimization

Fig. 1 shows the flow injection hydride generation metal furnace atomic absorption spectrometry system (FI-HG-MF-AAS) used in this work. The method was based on that previously proposed by González et al. [2] for As speciation, however substituting the metal furnace for the quartz tube atomizer (QTA). Carrier solution and flow rate, dilution media, reaction coil length, THB concentration and argon flow were optimized. The Inconel® tube atomizer as well as the flame condition (10 L min^{-1} air and 3.0 L min^{-1} acetylene flow rates) was previously optimized by Klassen et al. [9].

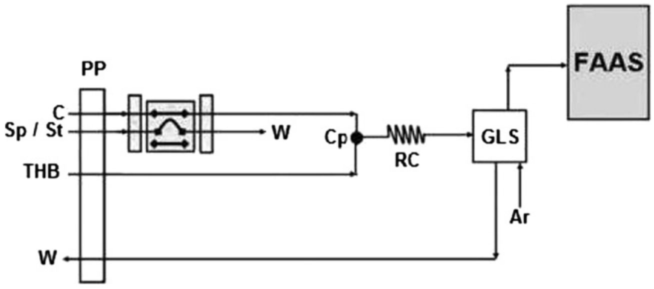

Fig. 1. FI-HG-MF-AAS system used in this work: peristaltic pump (PP); carrier stream (C); sample/standard (Sp/St); tetrahydridoborate(1−) (THB); waste (W); confluence point (Cp); reaction coil (RC); gas–liquid separator (GLS); and argon flow (Ar).

2.4. Pre-reducing agent optimization

Some pre-reducing agents were tested to reduce iAs(V) to iAs(III) in reaction media such as 2% (w/v) KI plus 4% (w/v) ascorbic acid [9], 2% (w/v) sodium thiosulfate [15], 2% (w/v) hydroxylamine hydrochloride [3], 2% (w/v) L-cysteine [1] or 2% (w/v) thiourea [16]. The inorganic As(III):As(V) ratio was estimated by measuring the electric potential of media containing the pre-reducing agent by using the Nernst equation, and comparing the resulting potential with data obtained by FI-HG-MF-AAS.

2.5. Accuracy test

This test was carried out with and without pre-reducing agent to verify the accuracy of the proposed method using NIST 1643e, trace elements in water, which presents 60.45 μg L^{-1} as total As.

2.6. Microwave-assisted extraction

To check the MAE efficiency for total As determination, *ca.* 100 mg of BCR414 material were weighted in three different PTFE flasks and 10 mL of deionized water were added to each one. To another nine flasks, deionized water was added as blank, since 12 flasks is the total capacity of the microwave oven used. The vessels were closed and a program with three extraction steps used as follows: 200 W for 2 min; 800 W for 10 min and 0 W for 30 min. Then, the flasks were cooled to room temperature, the extracts filtered, and volumes completed to 25 mL with deionized water. The total arsenic extracted was determined by GF AAS.

The same program was also used to verify the MAE efficiency for sample preparation and arsenic speciation. For this purpose, *ca.* 100 mg of BCR414 certified material was added to three flasks, 125 ng of iAs(III) was added to another three, 125 ng of iAs(V) to a further three flasks, and, finally, three had water as blanks. To each flask, 10 mL of deionized water was added and the extraction program applied, as described above. The flasks were then cooled to room temperature, the extract filtered, and volumes completed to 25 mL with deionized water. Both species, iAs(III) and iAs_t, were determined using the proposed FI-HG-MF-AAS system.

3. Results and discussions

3.1. Effect of the carrier

Air and a solution of 2% (v/v) HCl were evaluated as carriers for the system. For this purpose, analytical curves were obtained, and the results are shown in Fig. 2. Comparing Fig. 2a and b, a higher sensitivity and lower limits of detection and quantification, calculated according to the IUPAC recommendation [17], were obtained when air (Fig. 2b) was used as carrier. This is explained because the acid (as carrier) decomposes THB, decreasing the real concentration of the reagent in the system. However, using air as carrier, the acid concentration is lower at the end of the process, which decreases THB decomposition. This fact allowed a better evolution of arsane even at lower arsenic concentrations, as demonstrated in Fig. 2b. Thus air was considered the best carrier and used thereafter for all studies.

3.2. Effect of sample/standard dilution media on hydride generation

Solutions of 2% (v/v) HCl and citrate buffer (20% w/v of citric acid) at pH 4.50, as proposed by González et al. [2], were evaluated. For HCl, the results are shown in Fig. 2b. For citrate buffer, they are presented in Fig. 3. Comparing Figs. 2b and 3, citrate buffer shows higher sensitivity, and better limits of detection and quantification for iAs(III), and no measurable signal for iAs(V). This can be explained using the reduction equations (Eqs. (1) and (2)).

$$H_3AsO_{4(aq)}+2BH^-_{4(aq)}+2H^+_{(aq)}+5H_2O_{(l)}\rightleftharpoons H_3AsO_{3(aq)}+2H_3BO_{3(aq)}+7H_{2(g)} \quad (1)$$

$$H_3AsO_{3(aq)}+6BH^-_{4(aq)}+6H^+_{(aq)}+15H_2O_{(l)}\rightleftharpoons AsH_{3(g)}+6H_3BO_{3(aq)}+21H_{2(g)} \quad (2)$$

Eq. (1) shows that the reactive specie for iAs(V) is actually arsenic acid. At pH 4.50, all iAs(V) is presented as dihydrogenarsenate (Figs. 4a and 5). This means that the reaction shown in Eq. (1) would not be probable, considering the Principle of Le Châtelier, because the arsenic acid concentration in the medium has decreased. On the other hand, iAs(III) still remains as arsenous acid (Fig. 4b), which, according to Eq. (2), is the species that reacts to form arsane. This reaction is then probable. Eq. (1) also shows that the reduction of inorganic arsenic is pH dependent. The E_H vs pH diagram of inorganic arsenic (Fig. 5) shows that the redox potential equilibrium decreases when the pH increases. This means that a powerful reducing agent is necessary to reduce iAs(V) to iAs(III), which refers to equilibrium parameters (i.e. thermodynamic ones). Additionally, D'Ulivo et al. [18] proposed a mechanism of hydride generation from iAs(V) which involves kinetics parameter. The mechanism shows that the reaction begins with a collision between arsenic acid and THB, which has a negative charge. However, iAs(V) at pH 4.50 also presents a negative charge. In this way, no effective collision between these species occurs, also hampering this reaction. For these reasons, the reduction of iAs(V) to iAs(III) at pH 4.50 by THB is neither thermodynamically nor kinetically probable.

The higher sensitivity obtained with citrate buffer could be explained because of the higher ionic strength of the media. The concentration of 20% (w/v) citric acid corresponds to 1.04 mol L^{-1}. At pH 4.50, the ionic strength of the media is 1.9 mol L^{-1}. On the other hand, 2% (v/v) HCl is equivalent to 0.24 mol L^{-1}, and its ionic strength is also 0.24 mol L^{-1}. Ellis et al. [19] showed that by increasing the salt concentration an increase of arsane evolution is observed due to the salting-out effect. For all these reasons, citrate buffer was then chosen.

3.3. Effect of flow rate

The flow rate was evaluated from 3.5 to 17.4 mL min^{-1}. Increases of the signals were observed from 3.5 to 9.0 mL min^{-1} flow rate, because efficient reaction conditions were reached, probably due to the good mixing of the reagents inside the flow

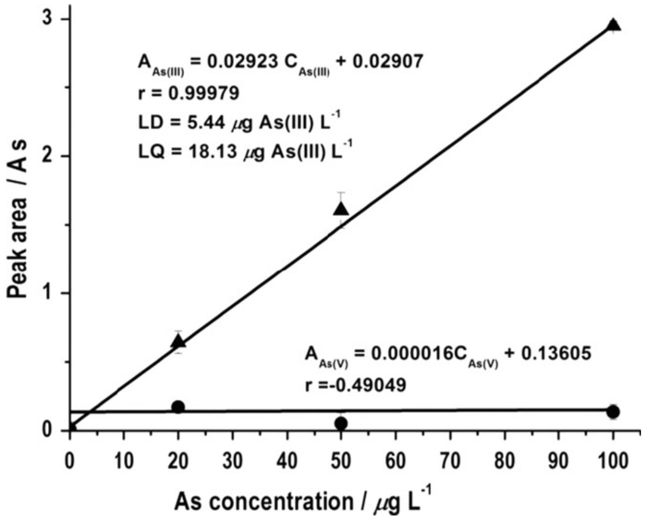

Fig. 3. Analytical curves using citrate buffer at pH 4.50 (in 20% w/v of citric acid) as dilution media (n=5 for each point). Fixed conditions: carrier: air; flow rate: 7.2 mL min^{-1}; THB: 0.05% (m/v); reaction coil length: 30 cm; argon flow rate in the GLS: 88 mL min^{-1}; injected volume: 1.6 mL ▲ iAs(III); ● iAs(V).

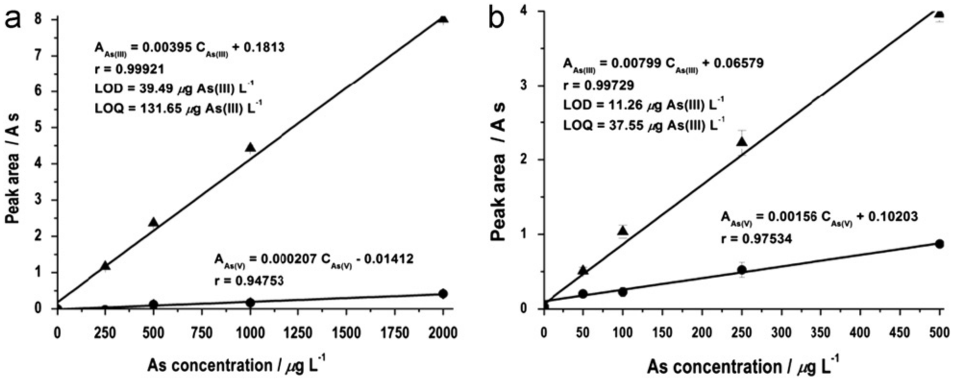

Fig. 2. Analytical curves using (a) 2% (v/v) HCl and (b) air as carrier (n=5 for each point). Fixed conditions: diluent media: 2% (v/v) HCl; flow rate: 7.2 mL min^{-1}; THB: 0.05% (m/v); reaction coil length: 30 cm; argon flow rate in the GLS: 88 mL min^{-1}; injected volume: 1.6 mL ▲ iAs(III); ● iAs(V).

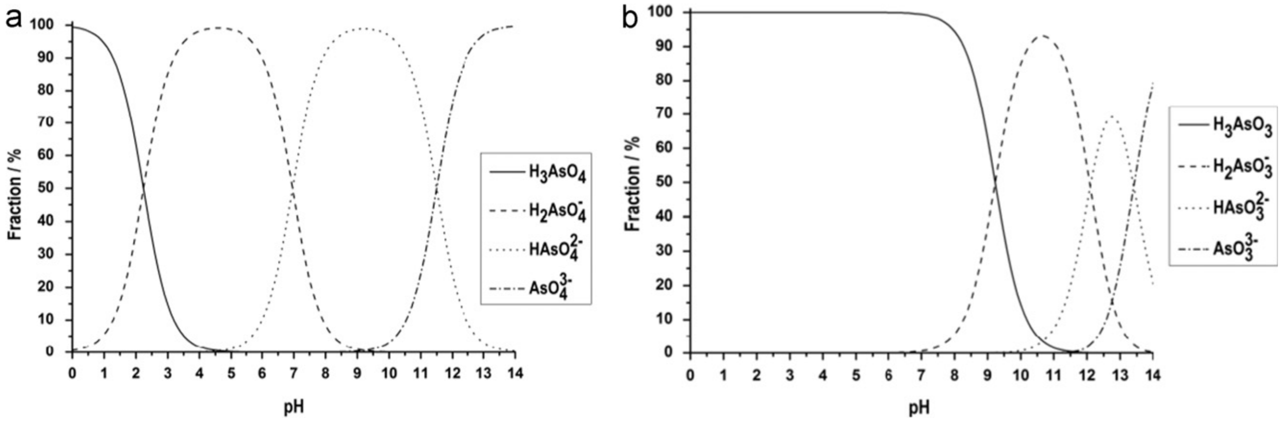

Fig. 4. Theoretical distribution of species by pH of (a) iAs(V) and (b) As(III).

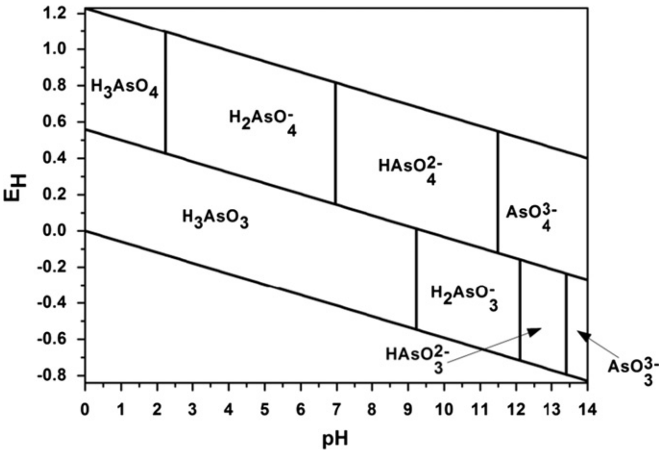

Fig. 5. E_H vs pH diagram for inorganic arsenic species.

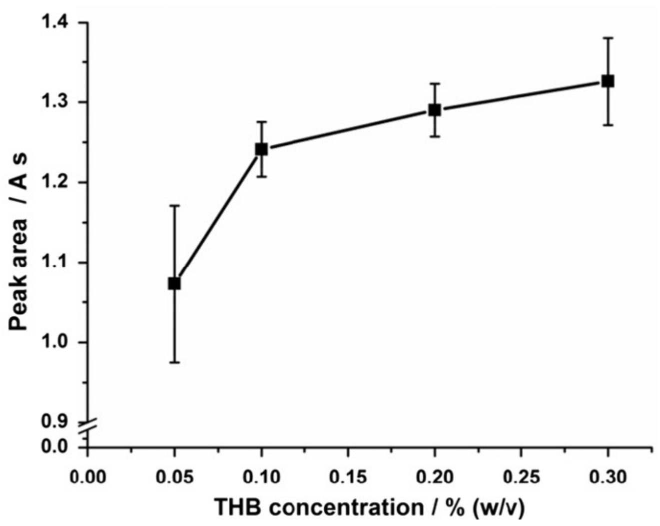

Fig. 6. Average analytical signal ($n=5$) for a 50 μg L^{-1} iAs(III) standard solution according to the concentration of THB. Conditions: carrier: air; diluent media: citrate buffer, pH 4.50; flow rate: 9.9 mL min^{-1}; reaction coil length: 120 cm; argon flow rate in the GLS: 88 mL min^{-1}; injected volume: 1.6 mL.

system. On the other hand, with flow rates higher than 10.0 mL min^{-1} the amount of the arsane was reduced and lower detectability observed, probably due to reduced efficiency in producing these mixing conditions. Even though good detectability for a 50 μg L^{-1} iAs(III) concentration was observed with 9.0 mL min^{-1}, the optimum flow rate was fixed to 9.9 mL min^{-1}, since no carryover effects were observed and the signal returns completely to the baseline after the measurement time (up to 60 s).

3.4. Effect of reaction coil length

The reaction coil length was evaluated with 30, 60, 90 and 120 cm. A solution of 50 μg L^{-1} iAs(III) was used for checking the best detectability. On the one hand it was observed that 90 cm produced the highest average signal (1.257 A s), on the other a larger dispersion of the results ($\sigma_{n-1}=0.139$) was noted. Coils with 60 and 120 cm length presented average signals of 1.200 and 1.224 A s, respectively, and quite similar dispersion of results (σ_{n-1} 0.091 and 0.093, respectively). Since smoothed signals were obtained with a 120 cm coil length, it was chosen for the next experiments.

3.5. Effect of sodium tetrahydridoborate(1−) (THB) concentration

The THB concentration was evaluated at 0.05, 0.1, 0.2 and 0.3% (w/v), and using a 50 μg L^{-1} iAs(III) concentration for checking the detectability (see Fig. 6). By increasing the THB concentration, the integrated absorbance (peak area) also increased. Although 0.30% (w/v) showed the highest signal, a poor signal profile was obtained. The explanation for this behavior is that the highest concentration of THB leads to higher H_2 generation due to THB decomposition. Then, the pressure inside the reaction coil increases, which contributes to increase the axial dispersion of the solution. This problem also happened at 0.20% (w/v). Then, 0.10% (w/v) was taken as the optimum condition and at this concentration good peak profiles were obtained.

3.6. Effect of argon flow rate in the gas–liquid separator

Also using 50 μg L^{-1} iAs(III) concentration, the argon flow rate in the GLS was evaluated at 88, 128 and 160 mL min^{-1}. When increasing argon flow rate from 88 to 128 mL min^{-1}, the analytical signal was also increased. This can be explained due to better mass transfer of the analyte from the system to the metallic furnace. However, at the highest argon flow rate tested, a decrease in the analytical signal was observed. In addition to mass transfer, Figueiredo et al. [7] showed that the temperature at the center of the Inconel 600® tube is slightly lower when compared to other regions. Then, at 160 mL min^{-1} the central region is cooler, thus impairing arsane atomization. This higher

Table 1
Potentials of pre-reducing agents.

Reducing agents (m/v)	***E*** **(mV)**	**A(III)/As(V) (*)**	**As(III) estimated (μg L^{-1}) (**)**	**As(III) found (μg L^{-1})**
2% KI+4% ascorbic acid	−72.0	4.41×10^{5}	50.00	< *LOQ*
2% $Na_2S_2O_3$	76.9	0.39×10^{1}	39.90	< *LOQ*
2% NH_2OH HCl	19.7	3.43×10^{2}	49.85	< *LOQ*
2% L-cysteine	−120.7	1.97×10^{7}	50.00	37.1 ± 1.9
2% thiourea	31.6	1.36×10^{2}	49.63	< *LOQ*

* Values estimated by Nernst equation.

** Considering 50 μg L^{-1} as total arsenic concentration and the ratio between As(III)/As(V).

flow rate might also cause the dilution of arsane in the gas phase, which could also contribute to a decreased signal.

After optimization, the *LOD* and *LOQ* of the proposed method were 2.0 and 6.6 μg L^{-1} of iAs(III), respectively, and the linearity ranged from 6.6 to 50 μg L^{-1}. Although these limits are higher than those achieved by González et al. [2] (0.07 and 0.24 μg L^{-1}, respectively), they are in accordance to the limits established by EPA [20] and WHO [21] for drinking water (10 μg L^{-1} total As as maximum).

3.7. *Effect of pre-reducing agent*

Some pre-reducing agents were tested to verify their efficiency in reducing iAs(V) to iAs(III) at pH 4.50. The potential of the solutions was measured with a redox electrode (Pt/Ag/AgCl, with saturated KCl gel as internal electrolyte) to estimate the iAs(III): iAs(V) ratio, using the Nernst equation. The results are shown in Table 1. The potential measured was corrected to the hydrogen potential scale before calculation, by adding 199 mV and converting the results to volts.

Table 1 shows that, except for $Na_2S_2O_3$, all the pre-reducing agents should be able to reduce iAs(V) to iAs(III) with a reduction efficiency close to 100%. Nevertheless, the experimental results show that only L-cysteine was able to reduce iAs(V), since, when employing the other reducing agents, signals lower than the *LOQ* were obtained. This behavior can be explained if the reduction kinetics is affected by pH, which was fixed at 4.50 for all experiments, as demonstrated in Section 3.2.

L-cysteine has a sulfhydryl group in its structure. Since iAs(III) has an strong affinity for this group, this interaction may change the mechanism of reduction of these species. In the same way, Gonzaléz et al. [2] successfully used thioglycolic acid to promote the reduction of As species, which also has a sulfhydryl group in its structure.

Concentrations of L-cysteine were evaluated from 0.5 to 5.0% (w/v) to verify the reduction efficiency. At 3% (w/v) of L-cysteine the recovery was already *ca.* 100%, and this concentration was chosen for next steps.

3.8. *Accuracy test*

This test was performed to verify the accuracy of the proposed method by using NIST 1643e certified material (trace elements in water) containing 60.45 μg L^{-1} of As. A 10 mL volume of sample was transferred to a 25 mL volumetric flask, mixed with 10 mL citrate buffer at pH 4.50 containing 50% (w/v) of citric acid, and the volume was completed with deionized water. This citric acid concentration was necessary in order to match the sample pH and ionic strength with the analytical standards. The results are shown in Table 2 and demonstrate that all the arsenic is probably present in the certified material as the pentavalent form.

Table 2
Results for As from NIST 1643e material using the proposed FI-HG-MF-AAS system.

	As concentration (μg L^{-1})
Certified value	60.45 ± 0.72
Value obtained without L-cysteine	< *LOQ*
Value obtained with L-cysteine	60.5 ± 3.3

Table 3
Potentials of the extracts obtained after microwave heating.

Extract	***E*** **(mV)**	**As(III):As(V) ratio(*)**	**As(III) expected (μg L^{-1}) (**)**	**As(III) found (μg L^{-1})**
As(III) standard	256.9	3.93×10^{-8}	1.31×10^{-14}	20.6 ± 2.4
As(V) standard	250.0	6.15×10^{-8}	2.05×10^{-14}	< *LOQ*
Plankton	238.1	1.71×10^{-7}	5.70×10^{-14}	< *LOQ*

* Values estimated by Nernst equation.

** Considering 25 μg L^{-1} as total arsenic and the ratio between As(III)/As(V).

Table 4
Recovery test for inorganic arsenic species in an aqueous standard using MAE.

Specie	**% recovery without L-cysteine**	**% recovery with 3% (w/w) L-cysteine**
iAs(III)	81.6 ± 0.1	114 ± 10
iAs(V)	< *LOQ*	96 ± 13

3.9. *Microwave-assisted extraction*

The recovery of total arsenic in certified material (BCR414, plankton), obtained through GF AAS analysis, was 94.0 ± 2.3%. Raab et al. [22] has already made an inter-laboratory trial looking for identification and quantification of water-soluble species in several seaweed samples, and no laboratory obtained 100% recovery although they did not look for “insoluble” arsenic species. In BCR414 *ca.* 0.41 μg g^{-1} could be considered as “insoluble species”. Although the material certification does not bring any information if it is phytoplankton or zooplankton, or even a mixture of both, it is possible to assume some similarity with seaweed.

Using the proposed FI-HG-MF-AAS for iAs(III) determination in plankton and iAs(V) standard solution extracts, the recovery was below the *LOQ*, meaning that the certified material has no extractable trivalent inorganic arsenic in its composition, and microwave heating itself is not able to reduce pentavalent inorganic arsenic. For iAs(III) standard solution extraction, the recovery was 81.6 ± 0.1%, showing that some iAs(III) loss occurred during the microwave extraction step, which may be attributed to the transfer process. The potential of these solutions was measured with a redox electrode (Pt/Ag/AgCl) to estimate the theoretical iAs(III):iAs(V) ratio. Table 3 shows that after the extraction, iAs(III) should be almost completely oxidized to iAs(V). Nevertheless iAs(III) was quantified in the solution extract [20.6 ± 2.4 μg L^{-1} iAs(III)], demonstrating that iAs(III) must be kinetically stable, even during closed vessel microwave extraction, since oxidizing conditions were attained even using water for microwave extraction [23].

The procedure was repeated for the determination of total inorganic arsenic, adding 3% (w/v) of L-cysteine before determination. Recoveries of 96 ± 13%, 55 ± 10% and 114 ± 6% for iAs(V) solution, plankton and iAs(III) solution, respectively, were obtained. The recovery above 100% for iAs(III) may be explained by changes in the solubility of arsane from adding L-cysteine. A summary of the

Table 5
Results of chemical speciation of As in BCR414—plankton certified material.

	As concentration ($\mu g\,g^{-1}$)	% recovery
Certified value	6.82 ± 0.28	100
Total extractable As (MAE+GF AAS)	6.41 ± 0.16	94 ± 6
Extractable iAs(III) (MAE+FI-HG-MF-AAS)	< 1.65	< 24
Extractable iAs(V) (MAE+L-cysteine+ FI-HG-MF-AAS)	3.79 ± 0.67	55 ± 10

results can be seen in Tables 4 and 5. It is interesting to note that mass balances were not possible, which may be explained due to insoluble organoarsenic species, such as arsenosugars, arsenocholine and arsenobetaine (estimated between 0.95 and 2.62 $\mu g\,g^{-1}$ in the BCR414). These species may be present in the certified material, as reported by Raab et al. [22].

4. Conclusions

The initial purpose of this work was successfully attained, making possible inorganic arsenic speciation using hydride generation with a metallic atomizer. To the best of our knowledge, this is the first work in the literature regarding As speciation using hydride generation with metallic atomizers.

In terms of As determination, the success in As speciation was only possible by controlling the reaction medium (slightly acid pH media and low THB concentration) to avoid the reduction of iAs(V). Using low concentrations of salts in the reaction medium, the sensitivity of the method was increased by decreasing gas solubility and increasing the evolution of gases (salting-out effect).

In terms of sample preparation for As speciation, MAE using water showed to be an interesting method for sample preparation since the relatively mild extraction conditions allowed the kinetic stabilization of inorganic arsenic species in the solutions.

Although the present method does not match the detectability of those using QTA, the *LOQ* obtained is below the limit for drinking water. Due to the easy and inexpensive implementation of the system, with the tube furnace costing *ca.* US$ 7 and its lifetime of ≥ 2000 h, the proposed method is then recommended for iAs(III) and total iAs determinations.

Acknowledgments

The authors thank the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP, São Paulo, Brazil) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, Brazil) for financial support. They also thank Prof. Carol H. Collins for language assistance.

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