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Speciation analysis of inorganic arsenic by a multisyringe flow injection system with hydride generation–atomic fluorescence spectrometric detection

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Abstract

In this study, a new technique by hydride generation–atomic fluorescence spectrometry (HG–AFS) for determination and speciation of inorganic arsenic using multisyringe flow injection analysis (MSFIA) is reported. The hydride (arsine) was generated by injecting precise known volumes of sample, a reducing sodium tetrahydroborate solution (0.2%), hydrochloric acid (6 M) and a pre-reducing solution (potassium iodide 10% and ascorbic acid 0.2%) to the system using a multisyringe burette coupled with one multi-port selection valve. This solution is used to pre-reduce As(V) to As(III), when the task is to speciate As(III) and As(V). As(V) is determined by the difference between total inorganic arsenic and As(III). The reagents are dispensed into a gas–liquid separation cell. An argon flow delivers the arsine into the flame of an atomic fluorescence spectrometer. A hydrogen flow has been used to support the flame. Nitrogen has been employed as a drier gas (Fig. 1).

Several variables such as sample and reagents volumes, flow rates and reagent concentrations were investigated in detail. A linear calibration graph was obtained for arsenic determination between 0.1 and 3 $\mu g\,l^{-1}$. The detection limit of the proposed technique ($3\sigma_b/S$) was 0.05 $\mu g\,l^{-1}$. The relative standard deviation (R.S.D.) of As at 1 $\mu g\,l^{-1}$ was 4.4 % ($n = 15$). A sample throughput of 10 samples per hour was achieved. This technique was validated by means of reference solid and water materials with good agreement with the certified values. Satisfactory results for speciation of As(III) and As(V) by means of the developed technique were obtained.

Keywords: Multisyringe flow injection analysis (MSFIA); Arsenic; Speciation; Hydride generation; Atomic fluorescence

1. Introduction

The toxicity of arsenic depends strongly on its chemical forms. Inorganic arsenic species are more toxic than the organic ones [1]. Inorganic trivalent forms are more toxic than the pentavalent forms [2]. Therefore, it is necessary to determine the individual species in order to estimate its environmental impact and health risks [3,4]. Although a number of methods have been developed, the conversion of the arsenic to volatile derivatives using the hydride generation method followed by analysis with an element-selective detector has been the preferred technique [5,6]. The use of hydride generation (HG) can separate analytes from sample matrices, thereby reducing or eliminating potential chemical and spectral interferences [3,6]. In addition, the chemical interferences from transition metals can be minimized by using high acidity and low reductant concentrations [7]. Atomic fluorescence spectrometry (AFS) has been used for determination of hydride-forming elements because of its high sensitivity, wide linear range, ease of use and low cost [3,6,8]. The coupling of HG with AFS has been widely reported for arsenic determination and/or speciation [1,6,8–13].

Flow injection analysis (FIA) has proved to be a suitable technique for on-line analysis because of its low reagents and sample consumption, high sampling frequency and ease of automation. Furthermore, it allows the reduction of transition metal interferences in comparison with the batch systems. It is due to a shorter period of sample–reagents interaction. On the other hand, the use of a lower concentration of reductants decreases the formation of interfering precipitates such as borides. In addition, the reduction of the hydride-forming elements is fast and the reaction is

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completed before the reduction of the transition metal ion to the interfering species [1,4,14–18].

Multisyringe flow injection analysis (MSFIA) was recently presented as a flow injection technique which combines the advantages of flow injection analysis (FIA) with the versatility and robustness of sequential injection analysis (SIA) [19]. The main device is a multisyringe burette with four syringes, which are connected in block to the same stepper motor, allowing the simultaneously movement of them. At the head of each syringe is a three-way solenoid valve, which leads to a reduction of the sample and reagent consumption, due to the possibility of injecting reagents only at the precise moment to perform the analytical determination or returning them to the stock bottle when they are not needed, without interfering with the remaining channels [19]. The acquisition of three successive peaks with only one filling of the syringe, increases the sample frequency and reduces the time of analysis. In addition, MSFIA technique can be easily applied to in situ environmental monitoring without demanding the continuous presence of the analyst. It helps to avoid the problems of sample preservation under field conditions, such as oxidation of As(III) [19]. MSFIA has been applied to the determination and pre-concentration of total inorganic arsenic by the authors [20,21].

The aim of this study is the development of a simple and sensitive procedure based on the applying of the MSFIA methodology coupled to an HG–AFS system for the determination and speciation of inorganic arsenic. Total inorganic arsenic was determined after on-line pre-reduction of As(V) to As(III) using potassium iodide. The concentration of As(V) was calculated by the difference of the total inorganic arsenic and As(III). Ascorbic acid was added to potassium iodide in order to avoid the oxidation of iodide to free iodine by the oxygen present in the aqueous streams [15,17,22,23]. The system has been optimized by changing different parameters such as sample and reagents volumes and flow rates, reagent concentrations, pre-reduction time, gases flow rates, among others.

2. Experimental

2.1. Reagents

All chemicals used were of analytical reagent grade. Millipore purified water (18.2 MΩ cm) was used for all solution preparation. Stock solutions 1000 mg l^{-1} arsenic(III) and arsenic(V) were prepared by dissolving 1.320 g As_2O_3 (Merck) and 1.534 g As_2O_5 (Aldrich) in 1000 ml of 0.1 mol l^{-1} NaOH solution. These stock solutions were kept at 4 °C in darkness. Working solutions of arsenic (0.1–3.0 μg l^{-1}) were prepared daily by dilution of the stock solution with HCl 4 mol l^{-1} (37%, Riedel-deHaën). Hydrochloric acid 6 mol l^{-1} was prepared from concentrated HCl (37%, Riedel-deHaën). The stock solution of 5.7–6.0% (w/v) $NaBH_4$ (Fluka, 97%) was prepared once a week by dissolving the appropriate amount of sodium tetrahydroborate in 0.2 mol l^{-1} NaOH (Merck). Working solutions of sodium tetrahydroborate were prepared daily by dilution of the $NaBH_4$ stock solution in Millipore water. Pre-reducing solutions containing 10% (w/v) potassium iodide (Fluka) and 0.2% (w/v) ascorbic acid (Fluka) were prepared fresh daily in water. Glassware used for the determination of As was soaked in a 10% (v/v) nitric acid solution and rinsed with Millipore water.

2.2. Apparatus

The MSFIA–HG–AFS system used for arsenic determination and speciation is shown in Fig. 1. The basic element is a

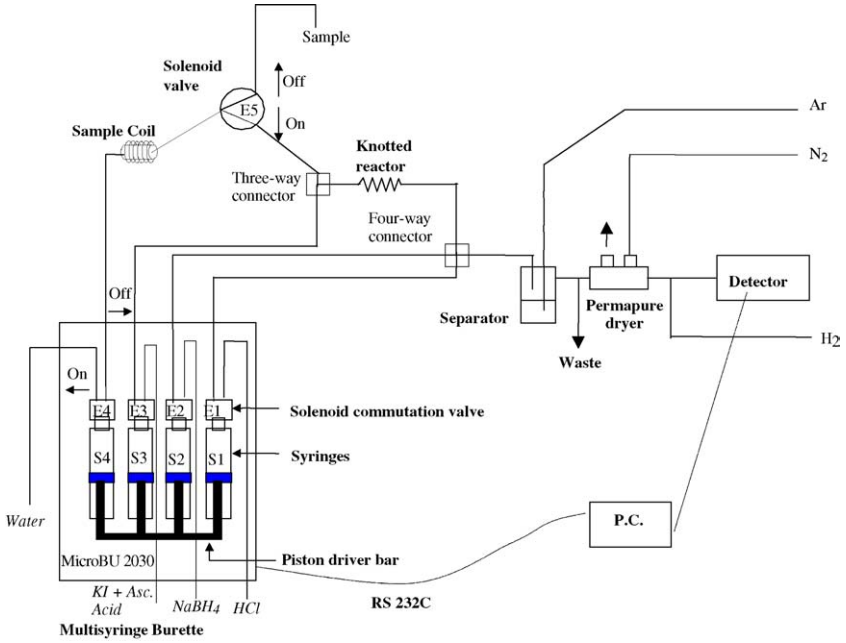

Fig. 1. MSFIA–HG–AFS system proposed for speciation and determination of As.

Table 1
Analytical procedure for speciation and determination of inorganic As

Step	Volume (ml)	Flow rate (ml/min)	Operation	Position of solenoid valves					Description
				E1	E2	E3	E4	E5	
1	0.6	7.7	Dispense	Off	Off	Off	Off	On	System and sample tube washing
2	0.4	7	Dispense	Off	Off	Off	Off	On	System and tube washing
3			Measure						PSA Excalibur Instrument
4	0.7	6	Dispense	On	On	On	Off	On	Blank injection and measuring (steps 3-5)
5			Wait 30 s						Wait until the reaction is fulfilled
6	0.7	7.7	Pick up	Off	Off	Off	Off	Off	Loading of syringes
7			Repeat three times from steps 3–6						
8	4	9	Dispense	Off	Off	Off	Off	On	System washing
9	4	9	Pick up	Off	Off	Off	Off	Off	Filling of tubes with sample and reagents
10	3.2	6	Dispense	Off	Off	Off	Off	On	Sample introduction to knotted reactor
11	3.7	6	Pick up	Off	Off	Off	On	Off	Loading of syringes
12	0.5	6	Dispense	Off	Off	Off	Off	On	Filling of tubes with reagents and sample
13			Measure						PSA Excalibur Instrument
14	0.7	6	Dispense	On	On	Off	Off	On	Sample injection and measuring As III
15			Wait 50 s						Wait until the reaction is fulfilled
16			Repeat three times from steps 13 to 15						
17	3.2	7.7	Pick up	Off	Off	Off	Off	Off	Filling of tubes with sample and reagents
18	3.2	6	Dispense	Off	Off	On	Off	On	KI-Sample introduction to knotted reactor
19			Wait 300 s						Pre-reduction time of As(V) to As(III)
20	3.2	7.7	Pick up	Off	Off	Off	On	Off	Loading of syringes
21			Measure						PSA Excalibur Instrument
22	0.7	6	Dispense	On	On	Off	Off	On	Sample injection and measuring total As
23			Wait 50 s						Wait until the reaction is fulfilled
24			Repeat three times from steps 21 to 23						
25	4	9	Pick up	Off	Off	Off	On	Off	Sample tube washing
26	4	9	Dispense	On	On	On	Off	On	Sample tube washing

E4 off/E5 on: dispense sample to the detector; E1 off: returns HCl solution to its bottle; E1, E2, E3 on: dispense reagents to the manifold; E4 on/E5 off: dispense sample to waste or stock bottle. The indicated volumes are referred to syringe 1.

multisyringe burette with programmable speed (MicroBU 2030, Crison, Alella, Barcelona). It consists of four syringes (5, 2.5, 2.5 and 5 ml) equipped with a three-way solenoid commutation valve on each head (E1, E2, E3, E4) (N-Research, Caldwell, NJ). The multisyringe burette also has one independent three-way solenoid commutation valve (E5) (N-Research, Caldwell, NJ).

The manifold was built from 1.5 mm i.d. (for picking up reagents) and 0.8 mm i.d. (to transport reagents and sample to the system) PTFE tubing. In order to improve the mixture of sample and pre-reducing solution, a knotted reactor (800 cm long) was made of a 0.8 mm i.d. PTFE tubing. A three-channel cross fitting is coupled to the pre-reducing solution tubing, the independent three-way solenoid commutation valve (E5) and the knotted reactor. An additional cross-shaped (four-way) connector is assembled to integrate the reagents tubing manifold and the knotted reactor to the glass gas–liquid separator.

A Permapure membrane (Perma Pure Inc., Toms River, NJ) was employed to remove the moisture of the gaseous phase before their introduction into the detection unit. Argon and nitrogen were used as a carrier gas and a dryer gas, respectively. An external cylinder of hydrogen was required to support the flame.

An atomic fluorescence spectrometer (10.044 Excalibur detector, PS Analytical) was employed for arsenic detection. It is equipped with an arsenic boosted discharged hollow cathode lamp (primary current 27.5 mA, secondary current 35.0 mA, wavelenght 193.7 nm) from Photron (Victoria, Australia). A fine gain of eigth was used for the analytical performance. The coarse gain setting was 100.

Instrumental control and data acquisition were performed using the software Autoanalysis[1] developed by our research group [24].

A 1200 Mega Milestone model mls microwave oven was used for sample digestion.

2.3. Procedure

The procedure used for inorganic arsenic determination and speciation is shown in Table 1. The MSFIA application was carried out using the four syringes (S1–S4) of the multisyringe burette with their corresponding solenoid commutation valves (E1–E4). The position “off” (solenoid disabled) of the valves connects syringes to the right channel and “on” (solenoid enabled) to the left one. One additional three-way commutation valves (E5) was coupled to the multisyringe burette. The

[1] The program may be requested at sciware_sl@yahoo.es; http://www.SCIWARE-SL.com.

syringe S1 (5 ml), which is connected to valve E1, contains the hydrochloric acid solution. The syringe S2 (2.5 ml), connected to valve E2, was used to impel the sodium tetrahydroborate solution. The syringe S3 (2.5 ml), with its corresponding valve E3, dispenses the pre-reducing solution to the knotted reactor through the three-channel cross fitting. The fourth syringe (S4, 5 ml), connected to valves E4 and E5, is used for loading and dispensing the sample (PTFE sample coil, 3.5 m long, 1.5 mm i.d.) to the knotted reactor. When the positions of valves E4 and E5 are "off", the sample is loaded (pick up operation). The position "E4 off/E5 on" indicates that the sample is dispensed to the detector, whereas position "E4 on/E5 off" dispenses sample to waste or stock bottle (dispense operation). Each channel is connected to a four-way-cross-shaped connector, which in turn is connected to the gas–liquid separator. Pre-reducing solution (KI–ascorbic acid) and sample are mixed in the knotted reactor, in which the on-line pre-reduction of As(V) to As(III) is carried out. After a determined pre-reduction time, sample, pre-reducing solution, hydrochloric acid and sodium tetrahydroborate solution are dispensed to the gas–liquid separator, generating arsine and achieving the acquisition of three successive peaks with only one filling of the syringe, increasing the sample frequency. In the first stage, As(III) is determined directly and immediately, whereas in the second stage, the total inorganic arsenic is carried out. As(V) is calculated by the difference between total inorganic arsenic and As(III). The indicated volumes in Table 1 are referred to syringe S1 (5 ml). It corresponds a half of the value for syringes S2 and S3.

2.4. Sample pre-treatment

The following certified reference materials were used to check the accuracy of the developed method: water samples, TMDA-54.3 (filtered and diluted Ontario lake water, National Water Research Institute, Canada) and CASS-4 (nearshore seawater, National Research Council Canada, Ontario, Canada); solid sample, CRM-279 (sea lettuce, BCR, European Commission, Belgium).

One and 7 ml of TMDA-54.3 and CASS-4 were diluted up to 50 and 10 ml, respectively, with 4 and 12 mol l^{-1} HCl. 0.5 g of CRM-279 was weighed and subjected to HNO_3 (65%, pro analysis, Merck)–H_2O_2 (35%, extra pure, Scharlau) digestion in a microwave oven with high-pressure pumps by the usage of a standard program from Milestone Cookbook. The sample was made up to 100 ml with Millipore water and then diluted (5 ml of the sample) to 50 ml with 4 mol l^{-1} HCl.

3. Results and discussion

3.1. MSFIA system

The parameters controlling each step of the analytical process have been optimized under the criteria of providing the best sensitivity and reproducibility as well as good recoveries for the analyte measurement in samples. After an initial assessment to select approximate values for each parameter, optimization of the variables was carried out by the univariate method.

The influence of the reaction coil, in which the on-line pre-reduction of As(V) to As(III) is carried out, was investigated. For this purpose, three different types of reaction coils were tested: coiled straight tube of 2.0 m (PTFE, i.d. 0.8 mm), coiled and knotted reactor of 2.0 m (PTFE, i.d. 0.8 mm) and coiled and knotted reactor of 8.0 m (PTFE, i.d. 0.8 mm). Conditions were following: 0.7 ml of 4 mol l^{-1} HCl (S1), 0.35 ml of a 0.2% (w/v) $NaBH_4$ solution (S2), 0.35 ml of a mixture of 5% (w/v) KI and 0.3% (w/v) ascorbic acid in Millipore water (S3) and 0.7 ml of 2 μg l^{-1} of As(III) and As(V) solutions in 4 mol l^{-1} HCl(S4); the pre-reduction time was 10 min. The recovery of As(V) was as follows: 48–55% for 2.0 m straight tube, 67–85% in the case of knotted reactor of 2.0 m and 97–100% for the 8.0 m knotted reactor. It confirms that knotted reactors provides a higher analytical sensitivity due to its three-dimensionally disoriented design, ensuring rapid mixing of sample and reagents solutions, enhancing radial mixing and decreasing axial dispersion of an injected sample [25,26]. The knotted reactor of 8.0 m (PTFE, i.d. 0.8 mm) was chosen to carry out the pre-reduction in further experiments.

The different volumes of the syringes were not tested in this study and the selection of them was based in a previous work [27].

As a carrier (in syringe 4), different HCl concentrations ranging from 1 to 4 mol l^{-1}, as well as water, have been evaluated and no significant differences were observed. In order to reduce the acid consumption, water was chosen as a carrier.

3.2. On-line reduction of As(V)

In order to ensure that the reduction of As(V) to As(III) is complete before hydride generation takes place, the effect of the pre-reduction time at room temperature was evaluated for 60, 120, 180, 300, 420, 600, 720 and 900 s. For this purpose, 0.7 ml of 2 μg l^{-1} of As(III) (in the first stage) and As(V) (in the second stage) solutions in 4 mol l^{-1} HCl (S4) and 0.35 ml of a mixture of 5% (w/v) KI and 0.3% (w/v) ascorbic acid in Millipore water (S3) were dispensed to the knotted reactor (PTFE, 8 m, i.d. 0.8 mm). After a predetermined period of time, both, pre-reducing and arsenic solutions were dispensed to the system with the rest of the reagents (0.7 ml of 4 mol l^{-1} HCl (S1) and 0.35 ml of a 0.2% (w/v) $NaBH_4$ solution (S2)), generating arsine. The recovery of As(V) was completed in 600 s. Promoting the reduction of As(V) to As(III) in a shorter time by increasing the pre-reductor concentration, the effect of the KI concentration was studied in a wide range from 0 to 15% (w/v) KI and 0.3% (w/v) ascorbic acid in 300 s predetermined reduction time. A KI concentration of 10% was enough to complete pre-reduction of As(V) to As(III) in 300 s.

The optimization of ascorbic acid was carried out under the same conditions mentioned above. Apparently, ascorbic acid concentration has no influence on the analytical performance. Therefore, the role of ascorbic acid is primarily to stabilize the pre-reduction medium. The concentration of 0.2% (w/v) ascorbic acid was chosen as optimal value.

The successive experiments were carried out using a pre-reducing solution of 10% (w/v) KI and 0.2% (w/v) ascorbic acid.

The pre-reduction time was 300 s, being a reasonable compromise.

Several researchers, each using their own procedure, came to different conclusions concerning the reduction of As(V): Näykki et al. [16] reported a pre-reduction time of 15 min and a mixture of 0.3% KI–0.8% ascorbic acid for 1 μg l^{-1} As(V) solution. Nielsen and Hansen [15] used 2% KI–1% ascorbic acid to reduce 5 μg l^{-1} As(V) in 60 s, heating the reduction medium at 140 °C and then cooling at 10 °C. Shraim et al. [28] reported a reduction time of 8–10 min for 40 μg l^{-1} As sample using 4.0% L-cysteine as a reduction agent. 5% L-cysteine decreased the reduction time to 300 s. Carrero et al. [29] reported 30 min for the pre-reduction of four arsenic species in 20 μg l^{-1} As sample with 1% L-cysteine. A mixture of 2.5/0.25 mg/ml sodium *meta*-bisulfite/sodium thiosulfate reduced As(V) in 420 s for a 180 μg l^{-1} As sample [30].

3.3. Optimization of volumes and flow rates

The influence of sample and reagents volumes on the efficiency of arsine generation was assessed over the 0.1–1 ml range (Fig. 2a). For this purpose, 1 μg l^{-1} of As(III) and As(V) solutions in 4 mol l^{-1} HCl, a mixture of 10% (w/v) KI and 0.2% (w/v) ascorbic acid in Millipore water, 4 mol l^{-1} HCl and 0.2% (w/v) $NaBH_4$ solutions were dispensed to the system. The pre-reduction time was 300 s. As can be seen in Fig. 2a, the sensitivity increased with increasing sample and reagents volumes. Nevertheless, higher volumes reduce sample frequency. Thus, a volume of 0.7 ml was selected as most convenient.

The effect of sample and reagents flow rates on the arsenic determination and speciation was investigated in the range from

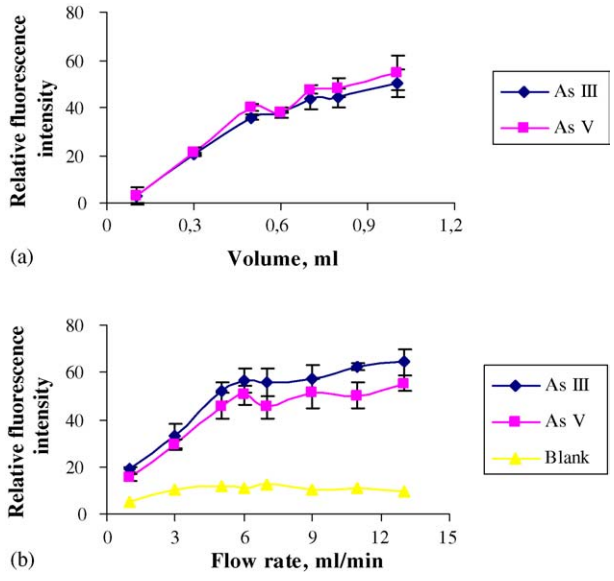

Fig. 2. (a) Effect of sample and reagents volume variation. Working conditions: 1 μg l^{-1} of As(III) and As(V) solutions in 4 mol l^{-1} HCl, 0.2% of $NaBH_4$, 4 mol l^{-1} HCl and 10% KI–0.2% ascorbic acid (error bars represent the standard deviation of triplicate analysis). (b) Effect of sample and reagents flow rate variation. Working conditions: 1 μg l^{-1} of As(III) and As(V) solutions in 4 mol l^{-1} HCl, 0.2% of $NaBH_4$, 4 mol l^{-1} HCl and 10% KI–0.2% ascorbic acid (error bars represent the standard deviation of triplicate analysis).

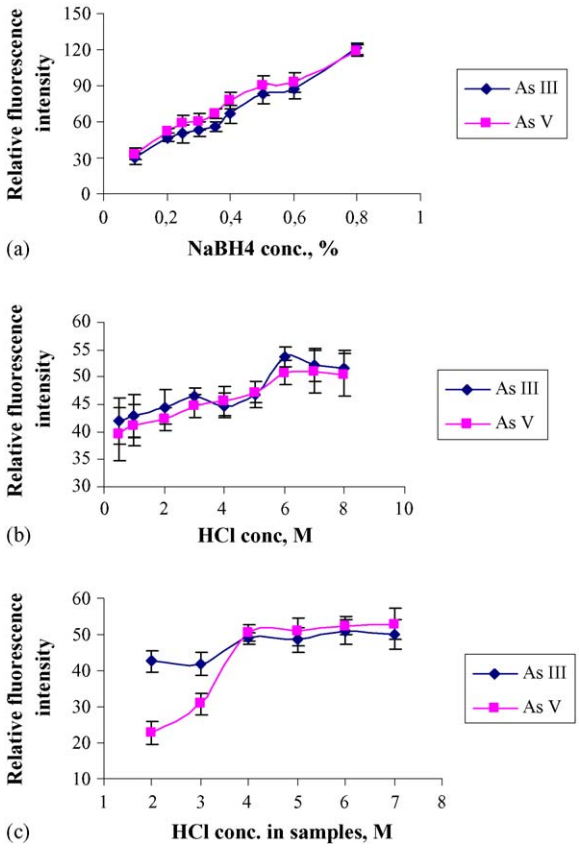

Fig. 3. (a) Effect of changing reducing agent concentration. Working conditions: 1 μg l^{-1} of As(III) and As(V) solutions in 4 mol l^{-1} HCl, 0.2% of $NaBH_4$, 4 mol l^{-1} HCl and 10% KI–0.2% ascorbic acid (error bars represent the standard deviation of triplicate analysis). (b) Effect of changing HCl concentration. Working conditions: 1 μg l^{-1} of As(III) and As(V) solutions in 4 mol l^{-1} HCl, 0.2% of $NaBH_4$, 4 mol l^{-1} HCl and 10% KI–0.2% ascorbic acid (error bars represent the standard deviation of triplicate analysis). (c) Effect of changing HCl concentration in sample. Working conditions: 1 μg l^{-1} of As(III) and As(V) solutions in 4 mol l^{-1} HCl, 0.2% of $NaBH_4$, 6 mol l^{-1} HCl and 10% KI–0.2% ascorbic acid (error bars represent the standard deviation of triplicate analysis).

1 to 13 ml min^{-1} under the same conditions mentioned above. The results are shown in Fig. 2b. The signal increases significantly with increasing flow rate from 1 to 5 ml min^{-1}, varying less from 6 to 13 ml min^{-1}. However, for higher flow rates, the reduction of As(V) to As(III) is incomplete, probably due to an inefficient mixing of sample and reductant [31]. Six milliliter per minute was chosen as optimal value. As filling operations of the syringe and washing steps do not influence arsenic determination, the rates for these operations were selected within the range of 6–9 ml min^{-1} in order to improve the sample throughput.

3.4. Effect of reagents concentration

To study the effect of varying $NaBH_4$ concentration on the signal intensity, amounts of reducing agent ranging from 0.1 to 0.8% (w/v) $NaBH_4$ was evaluated (Fig. 3a). Conditions were following: 0.7 ml of 1 μg l^{-1} of As(III) and As(V) solutions in 4 mol l^{-1} HCl, 0.35 ml of a mixture of 10% (w/v) KI and 0.2% (w/v) ascorbic acid in Millipore water, 0.7 ml of 4 mol l^{-1}

HCl, 0.35 ml of different $NaBH_4$ concentrations, 6 ml min^{-1} sample and reagents flow rate and a 300 s pre-reduction time. It was found that by increasing the concentration of $NaBH_4$ the height peak was in turn increased. Nevertheless, the blank signal increased simultaneously. A 0.2% (w/v) $NaBH_4$ concentration provided the greatest ratio between As/blank signals and has been chosen for further experiments.

The influence of acid concentration under the conditions described above is shown in Fig. 3b. The range of HCl concentrations injected from syringe 1 (S1) was from 0.5 to 8 mol l^{-1}. The concentration of 6 mol l^{-1} was selected in order to obtain the best analytical performance.

The optimization of the sample HCl concentration in the presence of 10% (w/v) KI was carried out operating under the same conditions, except for the HCl concentration of syringe 1, fixed in 6 mol l^{-1}. As can be seen in Fig. 3c, the signal of As(V) increases significantly with increasing HCl concentration from 2 to 4 mol l^{-1}, remaining stable from 4 to 7 mol l^{-1}. It confirms that potassium iodide can reduce arsenic only in a strong acidic media [32,33,23,17]. The concentration of 4 mol l^{-1} was chosen as optimal value.

3.5. Influence of Ar, H_2 and N_2 flow rates

The effect of varying the gases flow rates on the analytical signal was evaluated under the following conditions: 0.7 ml of 6 mol l^{-1} HCl (S1), 0.35 ml of a 0.2% (w/v) $NaBH_4$ solution (S2), 0.35 ml of a mixture of 10% (w/v) KI and 0.2% (w/v) ascorbic acid in Millipore water (S3) and 0.7 ml of 1 μg l^{-1} of As(III) and As(V) solutions in 4 mol l^{-1} HCl (S4); the pre-reduction time was 5 min.

The influence of argon flow rate on the arsenic determination and speciation is shown in Fig. 4. The increase of the argon flow rate increases peak signal of As. An explanation of this behavior was given from Mester and Fodor [34]. By diluting the flame with argon, the amount of atoms per unit volume decreases, therefore the effect of self-absorption of the fluorescence moves to a higher concentration range. On the other hand, by increasing the argon flow rate, the flame temperature decreases, resulting in a smaller atom density, helping in the atomization process. However, it can be observe that flow rates higher than 250 ml min^{-1} do not increase the peak signal. Burguera et al. [31] mentioned

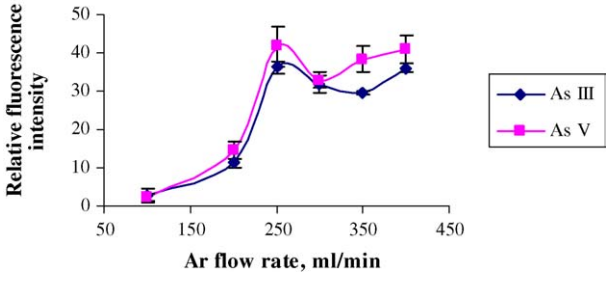

Fig. 4. Effect of changing Ar flow rate. Working conditions: 1 μg l^{-1} of As(III) and As(V) solutions in 4 mol l^{-1} HCl, 0.2% of $NaBH_4$, 6 mol l^{-1} HCl and 10% KI–0.2% ascorbic acid (error bars represent the standard deviation of triplicate analysis).

Table 2
Optimal operating conditions

Parameter	Value
Volume of sample and reagents (ml)	0.7
Flow rate of sample and reagents (ml min^{-1})	6
KI–ascorbic acid concentration (%) (w/v)	10% KI–0.2% ascorbic acid
$NaBH_4$ concentration (% (w/v))	0.2
HCl concentration (mol l^{-1})	6
HCl concentration in sample (mol l^{-1})	4
Pre-reduction time (s)	300
Flow rate of Ar (ml min^{-1})	250
Flow rate of H_2 (ml min^{-1})	55
Flow rate of N_2 (ml min^{-1})	1500
Fine gain	8

that as larger is the carrier flow rate the lesser is the residence time of the analyte in the atomizer, resulting in a loss of resolution, affecting the sensitivity. The best peak signal was obtained when the flow rate was 250 ml min^{-1}. It was selected as optimal value.

The effect of changing the flow rate of hydrogen was also investigated within the range 50–80 ml min^{-1}. It was found that decreasing H_2 flow rate, signal of sample was more stable and the ratio between As/blank signals was improved. A flow rate of 55 ml min^{-1} was chosen as most convenient. The authors observed in a previous work [20] that the flow rate of nitrogen used for drying purposes did not influence the value of the signal.

The optimal operation conditions are summarized in Table 2.

3.6. As(III) and As(V) recoveries

In order to evaluate the ability of the proposed technique to determine total inorganic arsenic, recoveries of separately prepared As(III) and As(V) solutions were investigated under the selected conditions summarized in Table 2. Working solutions of 0.5, 1.0 and 2.0 μg l^{-1} of As(III) and As(V) were prepared from As_2O_3 and As_2O_5, respectively. Recoveries obtained were in the range 99–102% for As(III) and 98–103% for As(V). These results confirm that the employed technique can be applied to total inorganic arsenic determination.

3.7. Analytical parameters

The analytical curve was obtained with As(V) standards within 0.1–3.0 μg l^{-1}. Typical regression line between relative fluorescence intensity and arsenic concentrations was described by the equation: $IF = 9.54 + 44.276\, C_{As}$ (μg l^{-1}) with a correlation coefficient of 0.994. Peaks corresponding to As standards were obtained by carrying out cycles of three injections for each standard solution. The relative standard deviation (R.S.D.), evaluated from 15 successive injections of 1 μg l^{-1} As standard solution, was 4.4%. The detection limit achieved is 0.05 μg l^{-1}. It has been calculated from three times the standard deviation of 15 blank signal measurements divided by the slope of the calibration curve ($3\sigma_b/S$). A sample throughput of 10 h^{-1} was obtained.

Table 3
Tolerance level of metal ions in the determination of 1 μg l^{-1} As(III)

Ion added	Fe	Cu	Pb	Cr	Co	Ni	Zn	Hg	Cd	Se	Sb
Tolerated concentration (μg l^{-1})[a]	200	1000	1000	200	1000	1000	25	700	25	400	5

[a] Ions do not interfere for concentrations (respect to 1 μg l^{-1} arsenic) up to these values.

3.8. Interferences

The study of the coexisting ions interferences on the determination of 1 μg l^{-1} As standard solution was carried out (Table 3). In these assays 1 μg l^{-1} As(III) in 4 mol l^{-1} HCl and KI–ascorbic acid solution were used for the experiments. An element was considered not to interfere if the highest peak variation is three times less than the standard deviation. As can be seen in Table 3, antimony produced the highest suppression on the signal. As Sb is a hydride-forming element, it can compete with arsenic for the reductant during the hydride generation step. Therefore, antimony can suppress the signal by affecting the generation of arsine even if its concentration is as low as that of the analyte [35]. On the other hand, the use of a higher concentration of reductant was avoided since it increases the blank signal, affecting the sensitivity of the technique.

Although a flow injection technique shortens the sample–reagents interaction period, diminishing the magnitude of the interferences, low tolerance levels of transition metal such as Zn and Cd have been obtained. It can be due to the adsorption of the analyte hydride onto metal colloids that are formed by reduction [18]. Moreover, the use of long reaction coils increases the residence time in the system. It could result in a lower tolerance level of metal ions [31].

On the other hand, it was found higher tolerance levels of Co, Ni and Cu in comparison with those obtained in previous works (with off-line pre-reduction) except for Fe, Zn and Cd [20,21]. Stockwell and Corns [7] reported that interferences from transition metal ions such as Co, Ni and Cu can be minimized by using high acidity and low reductant concentrations. Nielsen and Hansen [15] have been described a high threshold of tolerance of Cu and Co due to the high hydrochloric acid concentration, whereby the interferents form stable chloro complexes. Further, the presence of iodide and chloride might to some extent cause precipitation of Cu(II) and Co(II). In this study, we used a higher HCl concentration than in those works previously carried out by the authors [20,21].

Welz and Sucmanová [32] studied the influence of Ni and Cu on arsenic determination and found that the addition of potassium iodide-ascorbic acid to the As solution had a significant releasing effect on the nickel and cooper interferences, acting not only as a reductant, but also as a masking agent and interference releaser. They reported that the tolerance limit was increased by almost one order of magnitude. For our arsenic determination and speciation technique, we employed a concentration of KI 10 times higher than that reported previously, obtaining a higher tolerance level of Ni and Cu [20,21].

Yan et al. [1] reported that 100-fold excess of Cu, 1000-fold excess of Fe, Ni and Hg interfered with the determination of As(III). Nielsen and Hansen [15] achieved a tolerated concentrations of 60, 160, 140 and 500 times higher than that of As(III) for Cu, Co, Ni and Se, respectively. Tesfalidet and Irgum [18] reported a tolerated concentrations of 200, 400, 500 and 600 μg l^{-1} for Ni, Cu, Co, and Fe, respectively, in the determination of 50 μg l^{-1} As(III). The results obtained in the determination of As(III) using a pH selective hydride generation technique showed a higher tolerance level for Cu, Fe and Ba, among others of 2000-fold excess. A 1000-fold excess was reported for Al, Co, Ni, Cr, Pb and 500-fold-excess for Mn and Hg, among others [23].

3.9. Validation of the proposed technique

The proposed technique was validated by the analysis of two certified water reference material (TMDA-54.3, filtered and diluted Ontario lake water and CASS-4, nearshore seawater) and one solid reference material (CRM-279, sea lettuce). Three replicates of each water sample and four replicates for the solid sample were analyzed (Table 4). As can be observed from the table, satisfactory results have been obtained applying the proposed technique to solid and water samples.

3.10. Speciation of arsenic(III) and arsenic(V) by the proposed technique

In order to evaluate the possibilities of speciation of As(III) and As(V) using the proposed technique, the developed program (Table 1) was applied. Mixtures of different concentrations of As(III) and As(V) solutions were prepared. As was described previously in Sections 2.3 and 3.2, As(III) determination is carried out immediately, followed by the total inorganic arsenic determination. The concentration of As(V) is calculated as the subtraction of the two signals (Table 5). The results show a recovery of 100–110% for As(V), 100–120% for As(III) and 100–110% for total inorganic arsenic. Burguera and Burguera [5] suggest that the formation of arsine from As(V) involves two steps in the reaction: the reduction of As(V) to As(III) and the

Table 4
Results of the analysis of reference materials using the proposed method[a]

Sample	Certified value	Obtained value
Solid reference material		
CRM 279 (mg As kg^{-1})	3.09 ± 0.20	3.13 ± 0.11 (n = 12)[b]
Water reference material		
TMDA-54.3 (μg As l^{-1})	45.30 ± 7.31	49.80 ± 2.5 (n = 9)[c]
CASS-4 (μg As l^{-1})	1.11 ± 0.16	1.04 ± 0.2 (n = 9)

[a] Mean + S.D.
[b] Four replicates. Three peaks for each replicate.
[c] Three replicates. Three peaks for each replicate.

Table 5
Results of the speciation of As(III) and As(V) by the proposed technique

Injected concentrations ($\mu g\,l^{-1}$), As(III)–As(V)	Obtained concentrations ($\mu g\,l^{-1}$), total As/recovery (%)	Obtained concentrations ($\mu g\,l^{-1}$), As(III)/recovery (%)
0.2–0.2	$0.4 \pm 0.05/100$	$0.2 \pm 0.06/100$
0.5–0.5	$1.1 \pm 0.10/110$	$0.6 \pm 0.05/120$
0.5–1.0	$1.6 \pm 0.10/106$	$0.6 \pm 0.08/120$
1.0–1.0	$2.1 \pm 0.28/105$	$1.0 \pm 0.24/100$
1.5–0.5	$2.1 \pm 0.19/105$	$1.6 \pm 0.16/106$

subsequent formation of AsH_3. The rates of the redox reaction are rather slow, therefore it appeared that it might be possible to differentiate between the two species provided that the first stage of the reaction is slower than the second, even at high pH values. It allows that the hydride generation from As(III) in presence of As(V) is carried out without interference from the later [28,31,33].

3.11. Comparison between different flow techniques: FIA, SIA, MSFIA and MSFIA-speciation using HG–AFS

Comparison of results obtained by the authors using the commercial FIA system (PS Analytical), SIA and MSFIA–HG–AFS systems (with speciation step and without it) has been done [20]. Results presented in Table 6 showed that MSFIA-speciation system provides the same detection limit as the commercial FIA system. Nevertheless, MSFIA-speciation system allows to decrease considerably the reagents and sample consumption in comparison with the FIA technique, from three times less consumption of KI to 80 times less consumption of $NaBH_4$ and eight times less sample volume. In comparison with SIA system, MSFIA-speciation decreases the detection limit in 13 times and increases the sample throughput in almost two times, due to the acquisition of three peaks of sample with only one filling of the syringes. However, SIA system offers a more wide linear range. Applying the speciation technique to an MSFIA system, the consumption of KI and HCl increases considerably and the sample throughput decreases three times in comparison with the conventional MSFIA system, due to the pre-reduction step of As(V).

Table 6
Comparison results obtained by multisyringe flow injection analysis (MSFIA) with speciation, commercial flow injection analysis (FIA)[a], multisyringe flow injection analysis (MSFIA)[b] and sequential injection analysis (SIA)[b] systems

Parameters	FIA w.s.	SIA w.s.	MSFIA w.s.	MSFIA speciation
Detection limit ($\mu g\,l^{-1}$)	0.05	0.67	0.07	0.05
Linear range ($\mu g\,l^{-1}$)	0.1–8	2.5–70	0.25–12	0.1–3.0
% R.S.D.	–	1.9	4.9	4.4
Sample throughput (h^{-1})	–	6	36	10
Injection throughput (h^{-1})	45	33	113	47
$NaHB_4$ concentration (%)	1.2	0.12	0.24	0.20
$NaHB_4$ consumption (ml/inj)	4.7	0.5	0.3	0.35
$NaHB_4$ consumption (mg/inj)	56.4	0.6	0.7	0.7
HCl concentration ($mol\,l^{-1}$)	3	3	4	6
HCl (ml/inj)	11.3	0.5	0.6	0.7
HCl consumption (mmol/inj)	34.0	1.5	2.4	4.2
KI concentration (%)	1	1	1	10
KI/inj (ml/inj)	11.3	0.5	0.3	0.35
KI consumption (mg/inj)	113	5	3	35
Sample volume/inj (ml/inj)	5.7	0.5	0.6	0.7

w.s.: without speciation.
[a] Results obtained by the authors using commercial system made by PS Analytical Ltd.
[b] Results obtained by the authors.

4. Conclusions

The time-based MSFIA–HG–AFS system proposed for on-line speciation and determination of inorganic arsenic has proved to constitute an effective approach, since it allows almost simultaneous determination of As(III) and As(V). Other techniques that can be used for inorganic arsenic speciation, such as pH selective hydride generation [23,31] require controlling the reaction pH conditions and the time of hydride generation, whereas IC techniques [36] need the implementation and maintenance of an ion chromatography system. The overall MSFIA technique is practical, simple and robust. The proposed method offers some advantages such as compactness, optimum sensitivity, high selectivity and decreasing of reagents and sample consumption, which leads to a lower waste generation. A minor drawback is that the method requires a high hydrochloric acid concentration to ensure effective reduction of As(V), but also it contributes to a higher tolerance levels of transition metals. The multisyringe module and the constructed manifold allow an efficient sample introduction whereas the implementation of a knotted reactor improves the on-line pre-reduction of As(V), avoiding the use of a heating unit. The proposed technique was successfully applied to the determination of arsenic in reference solid and water materials. Nevertheless, in samples with a great ratio As(V)/As(III), the capability of the method will be limited by the range of the AFS instrument.

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