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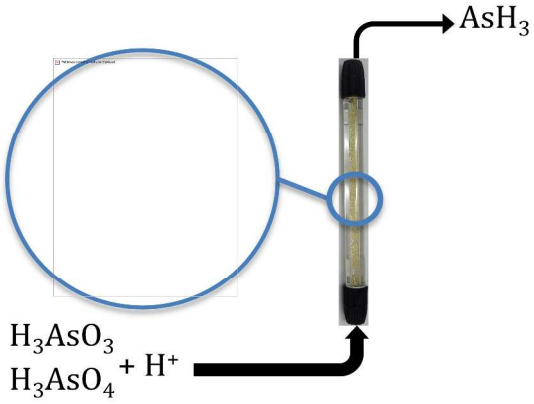
Non-chromatographic speciation of inorganic arsenic by atomic fluorescence spectrometry with flow injection hydride generation with a tetrahydroborate-form anion-exchanger

Nan Wang and Julian Tyson*

Two reaction conditions, with different HCl and L-cysteine concentrations, allow determination of both species in real tap, well, pond, and sea water samples down to 15 ng L^{-1}.



Non-chromatographic speciation of inorganic arsenic by atomic fluorescence spectrometry with flow injection hydride generation with a tetrahydroborate-form anion-exchanger†

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A method has been developed for the determination of arsenite and arsenate in natural water samples based on the generation of arsine (AsH_3) from the reaction between the arsenic species in the injected solution and tetrahydroborate immobilized on a strong anion-exchange resin (Amberlite IRA-400). Speciation was based on two different measurement conditions: (i) acidification to 0.7 M with HCl, and (ii) acidification to 0.1 M with HCl in the presence of 0.5% L-cysteine, which produced two calibration equations with different sensitivities for each species. The LOD for a 0.5 mL sample volume was 13 ng L^{-1} and 15 ng L^{-1} for arsenite and arsenate, respectively. The precision, expressed as % relative standard deviation of the measurement of 0.5 μg L^{-1} As was 4.3% and 4.1% for determination of arsenite and arsenate, respectively, in 0.7 M HCl; and 3.8% and 3.6%, for the determination in 0.1 M HCl and 0.5% L-cysteine. Interferences from transition metals and hydride-forming elements were eliminated by the addition of L-cysteine. The method was evaluated by the analysis of spiked natural waters. The recoveries for 0.5 and 1 μg/L arsenite were 92-108% and 88–112%, respectively; the recoveries for 0.5 and 1 μg/L arsenate were 94-111% and 95–112%, respectively. This method was also validated by the accurate analysis of a seawater certified reference material, NASS-6, which contains 1.43 ± 0.12 μg L^{-1} (total arsenic). The method was applied to the analysis of a number of real water samples, none of which contained arsenic below the method detection limit. The time required per measurement was less than 4 min and the procedure consumes about 100-times less hydrochloric acid that the conventional continuous-flow procedure.

Introduction

A major source of human exposure to inorganic arsenic is naturally contaminated drinking water from wells.[1] The resulting adverse health effects are a major concern for many countries, particularly those in SE Asia where thousands are predicted to die from arsenic-induced cancers.[2] Suitable analytical techniques are needed therefore to support studies of arsenic contamination, with a particular need for the rapid, accurate and low-cost analysis of groundwater. For these kinds of samples, inorganic arsenic species predominate and organic arsenic compounds are almost never encountered,[3] and so the need is for the determination of inorganic arsenic species.

In the determination of arsenic, hydride generation (HG) is a commonly used sample introduction technique for atomic spectrometry, as it not only enhances the atom number density significantly compared to those of nebulizer techniques but also separates the analyte from potential matrix interferences.[4] The technique also has potential for speciation analysis without chromatographic (or other real) separation of the analytes, as by adjusting the reaction conditions (principally the borohydride and acid solution concentrations), speciation can be achieved by the selective conversion of one species into a volatile hydride.[5] However, due to the instability of the aqueous borohydride solutions and the significant consumption of both borohydride and acid, there is interest in the generation of hydrides from solid reagents. Maleki *et al.*[6] used solid sodium borohydride and solid tartaric acid to generate plumbane. Tesfalidet and Irgum[7] first reported the generation of arsine with a column packed with an anion-exchange resin in the tetrahydroborate form. Tyson and coworkers have adapted this concept as the basis of flow injection atomic absorption spectrometry methods for the determination of cadmium,[8] antimony,[9] lead,[10] and selenium[11] with both quartz tube and graphite furnace atomizers. They also showed the potential for the speciation analysis of arsenic.[12] The relative sensitivities for arsenite and arsenate are closer when the anion-exchange procedure is applied[11] than is typically the case for flow injection HG in open tubular reactors.[13] The anion-exchange procedure not only decreases the consumption of reagents but also decreases matrix interferences, as the effective concentration of the borohydride is increased and the contact time between borohydride and

matrix components is decreased.

Atomic fluorescence spectrometry (AFS) is a viable detection technique for speciation studies concerning hydride-forming elements including arsenic.[14] A search of the "Web of Science" database for the past 6 years with "atomic absorption and arsenic", "atomic fluorescence and arsenic", "ICP MS and arsenic" in the title field shows that ICP-MS and AAS are the most popular techniques, with 65 and 86 publications in the database, respectively. Over the same time period, there were 56 publications describing the determination of arsenic by AFS. For the detection of arsenic, Heiler *et al.* found[15] that AFS was more precise, providing detection limits lower than those of AAS. AFS and ICP-MS have similar limits of detection for arsenic.[14,16,17] However AFS has advantages of much lower costs, and shorter warm-up times prior to analysis. Thus it is a viable alternative to ICP-MS or AAS, when low-cost, single-element speciation analysis with low-detection capability is needed.[16]

Our methodology for inorganic arsenic speciation is based that of Gonzalvez *et al.,*[18] who exploited the different behaviors of arsenic species in the HG reaction under two different conditions, combined with generation of arsine from reaction with a borohydride-form ion-exchanger. In general, the peak height of fluorescence intensities, I, measured under conditions A and B are related to arsenite and arsenate concentrations as follow:

$$I_A = \alpha_A + \beta_{A,III}C_{III} + \beta_{A,V}C_V$$

$$I_B = \alpha_B + \beta_{B,III}C_{III} + \beta_{B,V}C_V$$

where I_A and I_B are the peak height of fluorescence intensities under conditions A and B, respectively; C is concentration, α_A and α_B are the average intercept values of the linear calibrations for arsenite and arsenate under conditions A and B, respectively; β_A and β_B are the slopes of the linear calibrations obtained under conditions A and B, respectively; and the Roman numerals III and V indicate values for arsenite and arsenate, respectively. To create a second set of reaction conditions with different senstivities, Gonzalvez et al. added KI and ascorbic acid. In our work, we added L-cysteine, which is known to have a number of beneficial effects on the reaction between inorganic arsenic and borohydride.[19]

To the best of our knowledge, the application of immobilized tetrahydroborate for the determination of arsenic by AFS has not been previously reported. The goal was to develop a method for the accurate determination of inorganic arsenic species in natural water samples, and thus we have investigated the tolerance to interferences, measured the detection limit, and validated the method by the analyses of spiked samples and a standard reference material, NASS-6, for which we report the first speciation data. We applied the method to the analysis of a number of water (tap, well, pond and sea) samples, for none of which was the arsenic concentration below the LOD.

Experimental

Reagents

All chemicals were of analytical reagent grade. All solutions were prepared in 18 MΩ cm deionized water from a Barnstead E-pure system (Barnstead, Dubuque, USA). Solutions of sodium tetrahydroborate (98% purity, Alfa-Aesar, Ward Hill, MA) were freshly prepared daily by dissolving the appropriate amount of $NaBH_4$ in 0.5% (m/v) sodium hydroxide. The daily working standards for arsenic species were made from stock solutions (1000 mg L^{-1}) prepared from sodium arsenite ($NaAsO_2$) (Aldrich, Milwaukee, USA), sodium arsenate ($Na_3AsO_4{\cdot}7H_2O$) (Fisher Scientific, Pittsburgh, USA), disodium methyl arsenate [$(CH_3)AsO_3Na_2{\cdot}6H_2O$] (ChemService, West Chester, USA), and cacodylic acid [$(CH_3)_2AsO(OH)$] (Aldrich, Milwaukee, USA) by dissolving the accurately weighed solid material in deionized water. These stock solutions were kept at 4 °C in the dark.

Interference studies were carried out by adding stock salt solutions individually into arsenic-containing solutions. The salt stock solutions were prepared from ferric chloride ($FeCl_3$), manganese chloride ($MnCl_2 \cdot 4H_2O$), zinc nitrate [$Zn(NO_3)_2 \cdot 6H_2O$], cupric chloride ($CuCl_2{\cdot}2H_2O$), lead nitrate [$Pb(NO_3)_2$] (Fisher Scientific, Pittsburgh, USA), calcium chloride ($CaCl_2{\cdot}2H_2O$), magnesium chloride ($MgCl_2{\cdot}6H_2O$) (Alfa Aesar, Ward Hill, USA) and sodium

selenite (Na_2SeO_3) (Aldrich, St. Louis, USA). L-Cysteine (Aldrich, Milwaukee, USA) was added to all working standard solutions and samples at a final concentration of 0.5% (m/v). The resin was Amberlite IRA-400 (Aldrich, Milwaukee, USA), which is a strongly basic, anion-exchanger containing quaternary ammonium functional groups on a styrene-divinylbenzene structure. The certified reference material NASS-6 was purchased from the National Research Council Canada (Ottawa, Canada). Hydrogen and argon gas were delivered from compressed gas cylinders (Airgas, Salem, US).

Instrumentation

The atomic fluorescence spectrometer was a model Millennium Excalibur, (PS Analytical, Deerfield Beach, FL, USA), with a built-in Permapure dryer system (part number M025D002) and a gas–liquid separator (part number M055G003). The instrument was modified so that the flame was sustained by hydrogen from a cylinder rather than from the reaction of excess borohydride with acid in the continuous flow mode that is the normal operating procedure. Hydrogen gas was introduced through Teflon tubing into the system by merging with the purging argon gas before they were introduced into the gas-liquid separator. The hydrogen flow rate was controlled by a needle valve (Swagelok, Cleveland, US) and measured by a soap-bubble flow meter. The operating conditions are given in Table 1. Operation was controlled by Sams software (PS Analytical), which also recorded the transient signal that evolved after the acidified sample flowed though the anion-exchange column in the borohydride form. Peak height was measured and further data processing was done with Microsoft Excel.

The manifold, based on the design of Rodriguez and Tyson,[9] is shown schematically in Fig. 1. The column consisted of a glass tube of 60 mm length and 4 mm id, containing approximately 0.8 g of Amberlite IRA-400 resin, with glass wool packing at either end to prevent loss of resin and blockage of the connecting tubes. Other tubing was 0.8 mm id PTFE tubing. Before use, the freshly packed resin column was conditioned by washing several times alternately with 5% (m/v) borohydride and 0.1 M hydrochloric acid solutions. Two six-port rotary valves (Supelco,

Bellefonte, PA, USA) connected the column and the sample loop (500 μL) to the manifold. Three peristaltic pumps (two were built-in parts of the atomic fluorescence spectrometer, the other was from Cole Parmer, Vernon Hills, US), equipped with pump tubing of different internal diameters (Santoprene tubing with i.d. 1.85 mm for carrier and sample flow, Santoprene tubing with i.d. 1.30 mm for $NaBH_4$ flow and Tygon tubing with i.d. 2.78 mm for the drain) were used. Two of them controlled the flows of carrier and borohydride through the system; and the other one drained the waste from the gas–liquid separator. The three-step operating procedure[9] is shown in Fig. 1. In the load position, Fig. 1(a), the borohydride solution was pumped through the column for 60 s, converting the column to the borohydride form. At the same time, the carrier solution (deionized water) was pumped constantly through the system. Next, valve 2 was switched to the inject position, Fig. 1(b), and the column was washed with the carrier solution while the sample loop of valve 1 was filled. Finally, valve 1 was then switched to the inject position, Fig. 1(c), and the acidified sample was carried through the column to generate arsine. The optimum operating conditions, selected after the preliminary experiments, are given in Table 1.

Method development

Optimization

Although the figure of merit for the optimization process was maximum fluorescence peak height, boundary conditions relating to extinguishing of the flame, poor precision, and time of analysis were taken into account. The single-cycle, alternating-variable method was selected with peak height as the figure of merit to be maximized for the optimization, based on previous work.[10] Several iterations were made in order to establish the boundaries of the factor space. Results for the final cycle are shown. The effects of borohydride concentration, the time the borohydride solution was passed through the column, the flow rate of the borohydride and carrier solution, the carrier gas flow rate, the sample acidity, and the L-cysteine concentration on the signal were investigated for 0.8 g of resin in the column. The acidified sample flowed through

the column in the same direction as tetrahydroborate-loading solution. Dryer gas flow and lamp parameters were as recommended by the instrument manufacture. Since the method described here is not a continuous-flow method and uses much smaller amounts of HCl and $NaBH_4$, though does require an auxiliary hydrogen supply to sustain the flame. The hydrogen flow rate was chosen to match that produced in the conventional continuous flow hydride generation mode.

The effect of the borohydride concentration and sample acidity were studied by varying these parameters within the ranges 0.5–5% (m/v) $NaBH_4$ in 0.5% (m/v) NaOH, and 0.05–0.7 M HCl. The length of time that the borohydride was passed through the column was varied from 10 to 160 s. The effect of L-cysteine concentration was investigated within the range 0.1-2% (m/v) at 0.1 M HCl and 5% (m/v) $NaBH_4$. The borohydride and carrier flow rates were varied from 2.5 to 4.5 mL min^{-1}, and from 1.6 to 13.4 mL min^{-1}, respectively. Parameters were optimized for a sample solution of 1 μg L^{-1} arsenite. It is known that under flow injection conditions, the sensitivity for arsenite is greater than that of arsenate, and so it was expected that under whatever conditions were selected for the determination of arsenite, the sensitivity for arsenate would be lower. Even if the sensitivities were the same, this would not affect the ability of the procedure to distinguish between the two species as the basis of the method is that under a second set of conditions, the sensitivity of at least one species is different from that obtained under the first set of conditions.

Analytical performance

Under optimized conditions, calibration curves for 500 μL of 0.0, 0.3, 1.5, 3 and 6 μg L^{-1} of arsenic solutions in 0.7 M HCl, and in 0.1 M HCl and 0.5% (m/v) L-cysteine were constructed. Detection limits were calculated as the concentrations that gave signals equal to three times the standard deviations of 10 blank signals. The RSD of five replicate signals for solutions containing 0.5 μg L^{-1} of arsenic was calculated.

Interference studies

Interferences from a number of coexisting transition metals possibly present in natural water samples were investigated. The compositions of the natural water samples that were collected for this study were determined by ICP-MS, further details of which are provided in the supporting information. These metals were present in large excesses relative to the analytes. The concentrations of the potential interferences that were chosen for interference study were (a) similar to the metal concentrations found that were the highest, and (b) ten times these values.

In addition, the tolerances of the system to the hydride-active species selenite, MMA and DMA were studied. Selenite was added at concentrations that were 10 and 1000 times that of arsenic. The interferences were added individually to 1 μg L^{-1} of arsenic (arsenite) standard solution in 0.7 M HCl, or in 0.1 M HCl with 0.5% (m/v) L-cysteine. The responses of the system to monomethylarsonate and dimethylarsinate were also measured.

Analysis of water samples

Water from the Amherst town supply was collected, after running a tap in the laboratory for 5 min. Pond water was collected from the Campus Pond at the University of Massachusetts Amherst. The coastal seawater samples were collected at Provincetown, MA and Beverly, MA. Well water samples were collected from private wells located in and around Amherst, MA. For the same well, one sample was collected from the tap as the first draw in the morning and the other after the tap had been run for a few minutes. After delivery to the laboratory, samples were filtered through 0.45 μm hydrophilic filters (Millipore Corporation, Billerica, MA, USA) and stored at 4 °C. All samples were also analyzed by ICP-MS for elemental concentrations. Details are provided in the supporting information.

Results and discussion:

Parameter optimization:

The effects of (a) concentration of $NaBH_4$, (b) loading time of $NaBH_4$, (c) carrier flow rate, (d) sample acidity, (e) carrier gas flow rate, and (f) L-cysteine concentration are shown in Fig. 2.

Since the atomic fluorescence spectrometer is using a flame atomizer, a relatively stable gas flow is needed. As a result, the concentrations of HCl and $NaBH_4$ used in the HG reaction were restricted in order to product a moderate amount of H_2 gas so that the flame would not be disturbed (or extinguished). As shown in Fig. 2, the concentrations of HCl and $NaBH_4$ that gave maximum signals without extinguishing the flame were 0.7 M (Fig. 2d) and 5% (m/v) (Fig.2a), respectively. A concentration of 5% (m/v) $NaBH_4$, at a flow rate of 4.5 mL min^{-1}, and a loading time of 60 s were chosen as optimal. With greater amounts of borohydride on the column, the flame was unstable when the additional hydrogen was evolved. This could not be offset by decreasing the flow of hydrogen from the cylinder, as this was set at the minimum needed to sustain the flame. Studies on the effect of the carrier flow rate (Fig. 2c) showed an increase in the signal, as the carrier flow rate varied from 1.6 to 13.4 ml min^{-1}, and reached the maximum value at 7.5 ml min^{-1}.

Studies of the effect of argon flow rate, shown in Fig. 2e, showed that a maximum was obtained when the argon flow rate was set to a value of 250 ml min^{-1}, which is the same as suggested value by the manufacturer for conventional continuous flow HG. The slight decrease of fluorescence signal at higher carrier flow rate is considered to be the result of dilution in the gas phase.

The L-cysteine concentration was varied between 0 and 2% (m/v) in 0.1 M HCl. Fig.2f shows that 0.5% (m/v) L-cysteine, where the signal was maximized, was able to increase the arsenite fluorescence signal by a factor of about three.

The effect of acid concentration in the presence of 0.5% (m/v) L-cysteine was also studied. The fluorescence signal, which reached the highest value at only 0.1 M HCl, was even greater than the signal for 0.7 M HCl in the absence of L-cysteine. This means that less acid is consumed when L-cysteine is added. In addition, the background signal was lower. Under the optimized conditions, up to three measurements could be made before the column had to be reloaded with $NaBH_4$

Analytical performance:

The equations of the calibrations and the other performance figures of merit are summarized in Table 2. In the absence of L-cysteine (condition A), the sensitivity for arsenate was about 80% of that of arsenite, probably because of slower reaction kinetics, as the arsenate has to be reduced to arsenite by the borohydride before arsine is generated. This relative sensitivity is in accordance with the results of previous studies.[11,13] In the presence of L-cysteine (condition B) the sensitivities for the two species were the same, which is interpreted as the complete reduction of arsenate to arsenite. At the same time, the sensitivity for arsenite was increased by 21%. By combining the equations under each condition, the intensity (peak height in arbitrary units provided by the instrument software) under each condition is proportional to the concentration of arsenite and arsenate (in $\mu g\ L^{-1}$). Calibration curves, based on the measurement of 0, 0.3, 1.5, 3 and 6 $\mu g\ L^{-1}$ arsenite and arsenate standards, had the following equations:

$I_A = 61 + 192C_{III} + 150C_V$

$I_B = 48 + 232C_{III} + 233C_V$

As the calculation of the concentrations involves solving two simultaneous equations whose coefficients are experimental values (slopes and intercepts) derived from calibration functions, with associated uncertainties, the uncertainty in the concentrations will be greater than those that are calculated on the basis of a single calibration function. Although the statistical basis of accounting for the uncertainties in the calibration function is well known,[20] researchers usually present the results of replicate measurements of the samples rather than try to solve the equations involving the uncertainties in the slope and the intercept. One possible reason is that unless weighted regression is used, the ± terms associated with values close to the intercept are rather large. For the work reported here, the standard deviations of the intercepts are between 3 and 6 (arbitrary instrument response units) and for the slopes are between 1 and 2 [arbitrary instrument response units per ($\mu g\ L^{-1}$)]. When these uncertainties are incorporated into the expressions for the concentrations that are obtained from solving the two simultaneous equations, the ± terms associated with concentrations are between 2 and 4 times larger than the ± terms that would be

obtained from a calibration based on the response to a single species. A novel feature of the dual calibration approach is that three replicate measurements under the two conditions, allows each concentration to be calculated nine times, which provides a more realistic estimate of the uncertainty than is obtained by calculating the three values corresponding to matching the highest, the middle, and the lowest values. The uncertainties in the results are expressed as ± the 95% confidence interval based on nine values.

The LODs were 13 and 15 ng L^{-1} for arsenite and arsenate, respectively for a 500 µL sample volume. The precision, expressed as %RSD (n = 5), was 4.3%, and 4.1% for 0.5 µg L^{-1} arsenite and arsenate in 0.7 M HCl and 3.8% and 3.6% for 0.5 µg L^{-1} arsenite and arsenate in 0.1 M HCl with 0.5% L-cysteine, respectively. Under the conditions given in Table 1, the measurement time is just over 3 min per sample, comprising 60 s for column and sample loading, 5 s for the column rinse, 60 s for fluorescence detection, and 60 s for column wash.

Interferences

The results of the determination of the matrix elements showed that calcium and magnesium were two of the most abundant elements in the collected natural water samples, all of which were present at concentrations less than 10 mg L^{-1}. The interference effects of several coexisting elements at different concentrations in 0.7 M HCl and 0.1 M HCl with 0.5% (m/v) L-cysteine are shown in Table 3. No interference, up to 100 mg L^{-1}, was seen for calcium and magnesium. Zinc, manganese and lead did not affect the arsenic signal at concentrations of 0.001 and 0.1 mg L^{-1}. Iron(III) and copper suppressed the arsenic signal at concentrations of both 1 and 10 mg L^{-1}. The presence of copper resulted in 11 and 73% signal depressions at concentrations of 1 and 10 mg L^{-1}, respectively. In the presence of iron(III), the corresponding decreases in the signals were 32 and 53%. The interference effects of copper and iron on HG procedures are well known due to the competition between the analyte and the interferent metals for the borohydride and the decomposition of the arsine on the surfaces of metal and metal boride precipitates.[4] We have previously shown that the extent of interferences is decreased for the column reactor compared

with those observed in homogeneous solution.[10]

The hydride-forming element, selenium in the form of selenite affected the arsenic signal when added at concentrations of 1 and 10 mg L^{-1} when the arsenic signals were suppressed by 22% and 63%, respectively. Interferences from selenite can be attributed to competitive reactions in which the selenite competes with the arsenic species for sodium tetrahydroborate to form hydrides.[21,22 changed] When measured under the same condition (0.1 M HCl with 0.5% L-cysteine), the sensitivities for DMA and MMA are 50% and 17% of that for arsenite, respectively. The presence of these methylated compounds would constitute an interference, but as was pointed out in the introduction,[3] these compounds are not found in ground waters.

With the addition of L-cysteine, simultaneous signal enhancement and decrease of interferences has been observed previously for the determination of arsenic by other researchers.[19,23] In our study, employing L-cysteine decreased the interference effects of from Fe(III) and Cu(II) significantly as shown in Table 3. The mechanisms by which L-cysteine affects relevant HG (with borohydride) processes have been extensively studied by Brindle and coworkers.[19, 24] They showed that L-cysteine has a three-fold role in the determination of arsenic: arsenate is rapidly reduced at low acid concentrations, the signal is enhanced, and interferences are decreased.[19] They have also shown, in the determination of germanium, that borohydride reacts with L-cysteine to produce a more effective hydride transfer reagent, which is less reactive towards potentially interfering transition metals.[24] The reaction of borohydride with thiols to produce a reagent with more useful reactivity has been known since 1975,[25] though the nature of the reaction product was not elucidated until 1984.[26] This work was not cited by the relevant analytical chemistry community until 2004.[27] More recently, all of the relevant chemistry has been set out in an 2011 IUPAC Technical Report,[28] in which the additional possible benefits of the reaction between arsenite and L-cysteine to form thiol compounds of the form $As(SR)_3$ is considered. However, the reviewers conclude that it is the formation of the L-cysteine-borohydride complex is the key to sensitivity enhancement and interference control and that pretreatment to form arsenite-thiol complexes is not necessary.

It was also found that under condition B, at least five replicate measurements could be made without reloading the column; whereas under condition A, the number of replicates was only three. This is attributed to the lower concentration of acid for condition B, which results in less consumption of borohydride.

Determination of arsenic in natural waters

The applicability of the procedure is shown by the measurement of arsenic species in all of the water samples examined. The reliability of the procedure was confirmed by the analysis of spiked natural water samples and by the analysis of the certified reference material. The results are shown in Table 4 for the fresh water samples, from which it may be seen that the recoveries ranged from 88 to 112%, indicating that arsenic can be quantitatively recovered from various fresh waters.

However, the analyses of seawater was subject to more significant interferences such that low recoveries were obtained using external calibration with standard solutions, which could be a result of both the higher ionic strength and higher iron(III) concentrations of the seawater samples, as shown in Table S2 (supporting information). To overcome the interference problem, standard additions were employed. Multiplicative interferences were assessed by comparing standard and analyte addition calibration curves. Both analytical curves showed good linearity. The recoveries, which ranged from 94 to 98%, are shown in Table 5. The accuracy of the method was also verified by the analysis of certified reference material NASS-6 (seawater). The resulting values for the total arsenic concentration (1.40 ± 0.03 μg L^{-1}, n = 3, the ± term is one standard deviation) is not significantly different from the reference value of 1.43 ± 0.12 μg L^{-1}. The resulting value also indicates that the dominant detectable arsenic species are arsenate and arsenite, since methylated arsenic species, such DMA and MMA, are hydride active and interfere with the arsenic fluorescence signal. To the best of our knowledge, there is no report of the arsenic speciation in NASS-6. NASS-5, which has now been replaced by NASS-6, has been in the development of arsenic speciation methods.[29,30] Yip *et al.*[29] determined the arsenic speciation

by ion chromatography ICP-MS and found the concentrations of arsenite to be not detectable and that of arsenate to be 1.23 μg L^{-1}. Hsiung and Wang[27] found 0.44 μg L^{-1} arsenite and 0.84 μg L^{-1} arsenate in NASS-5 by cryogenic trapping HG-AAS. Although the results for the total arsenic content were not significantly from the reference value of total arsenic (1.27 μg L^{-1}), it can be seen that the speciation of arsenate and arsenite results were quite different, possibly related to oxidation during storage and/or preparation.

The standard addition method applied to the seawater samples could also be applied to fresh waters if interferences were significant. The metal species content of natural waters is correlated with both the composition of the sediment or mineral surface coatings[31] and microbial activity[32,33] and can be significantly different from place to place, so it is possible that a fresh water matrix could generate non-negligible interferences.

Conclusions

As for the previous methods involving HG from a borohydride-form anion exchanger,[8-10] the method for the determination of arsenic has the advantage of considerably decreased consumption of reagents compared with the consumption for the conventional continuous flow homogeneous reaction procedure. This is particularly true of the consumption of hydrochloric acid, whose consumption is decreased more than 100-fold. Details are provided in the supplemental information.

A popular speciation strategy is to find conditions that suppress the signal of one species completely, so that selective measurement of the other species can be made, and then to convert one species to the other (or choose conditions under which both species give the same response) so that a total measurement may be made. Such conditions may be difficult to establish. The speciation strategy adopted here only requires conditions under which the ratios of the sensitivities are different, which may be much easier to establish.

By coupling the borohydride-form anion exchanger HG system with AFS detection, a method was developed with sufficiently low detection limits for the determination inorganic

arsenic species at naturally occurring concentrations in several water samples. For only one of the samples examined was the concentration below either the method LOD and or the limit of quantification (defined as the concentration giving a signal equal to ten times the standard deviation of the signal for the blank). In addition, the method is not subject to the major interference from chloride that causes inaccuracies for ICP-MS (see Table S2 in the supplemental information). Work on extending the speciation methodology to include the methylated species is in progress.

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References

1 P. Ravenscroft, H. Brammer and K. Richards, *Arsenic Pollution: A Global Synthesis*, Wiley-Blackwell, Chichester, 2009.

2 D. C. Mukherjee, S. B. Chowdhury, A. Paul and P. Das, A review on arsenic pollution in West Bengal and Bihar : Cause, effects and remedial measures following latest technology. *J. Indian Chem. Soc*., 2012, **89**, 9-18.

3 I. Komorowicz and D. Barałkiewicz, Arsenic and its speciation in water samples by high performance liquid chromatography inductively coupled plasma mass spectrometry—Last decade review. *Talanta*, 2011, **84**, 247–261

4 J. Dedina and D. L. Tsalev, *Hydride Generation Atomic Absorption Spectrometry*, Wiley, New York, 1995.

5 A. R. Kumar and P. Riyazuddin, Non-chromatographic hydride generation atomic spectrometric techniques for the speciation analysis of arsenic, antimony, selenium, and tellurium in water samples - a review. *Int. J. Environ. Anal. Chem*., 2007, **87**, 469-500.

6 N. Maleki, A. Safavi and Z. Ramezani, Determination of lead by hydride generation atomic absorption spectrometry (HGAAS) using a solid medium for generating hydride. *J. Anal. At. Spectrom*., 1999, **14**, 1227-1230.

7 S. Tesfalidet and K. Irgum, Polymer-bound tetrahydroborate for arsine generation in a flow-injection system. *Anal. Chem*., 1989, **61**, 2079-2082.

8 W. Chuachuad and J. F. Tyson, Determination of cadmium by flow injection atomic absorption spectrometry with cold vapor generation by a tetrahydroborate-form anion-exchanger. *J. Anal. At. Spectrom*., 2005, **20**, 273-281.

9 Y. Rodriguez and J. F. Tyson, Determination of antimony by atomic absorption spectrometry with flow injection hydride generation by a tetrahydroborate-form anion-exchanger. *J. Anal. At. Spectrom*., 2006, **21**, 757-762.

10 W. Chuachuad and J. F. Tyson, Determination of lead by flow injection hydride generation atomic absorption spectrometry with tetrahydroborate immobilized on an anion-exchange resin. *J. Anal. At. Spectrom*., 2005, **20**, 282-288.

11 P. E. Carrero and J. F. Tyson, Determination of selenium by atomic absorption spectrometry with simultaneous retention of selenium(IV) and tetrahydroborate(III) on an anion-exchange resin followed by flow injection hydride generation from the solid phase. *Analyst*, 1997, **122**, 915-919.

12 J. F. Tyson, High-performance, flow-based, sample pre-treatment and introduction procedures for analytical atomic spectrometry, *J. Anal. At. Spectrom*., 1999, **14**, 169-178.

13 J. Dedina, D.L. Tsalev, *Hydride Generation Atomic Absorption Spectrometry*, Wiley, Chichester, 1995, pp. 182–245

14 D. Sanchez-Rodas, W. T. Corns, B. Chen and P. B. Stockwell, Atomic Fluorescence Spectrometry: a suitable detection technique in speciation studies for arsenic, selenium, antimony and mercury. *J. Anal. At. Spectrom*., 2010, **25**, 933-946.

15 J. F. Heilier, J. P. Buchet, V. Haufroid and D. Lison, Comparison of atomic absorption and

fluorescence spectroscopic methods for the routine determination of urinary arsenic. *Int. Arch. Occup. Environ. Health*, 2005, **78**, 51-59.

16 J. L. Gomez-Ariza, D. Sanchez-Rodas, I. Giraldez and E. Morales, A comparison between ICP-MS and AFS detection for arsenic speciation in environmental samples. *Talanta*, 2000, **51**, 257-268.

17 A. L. Lindberg, W. Goessler, M. Grander, B. Nermell and M. Vahter, Evaluation of the three most commonly used analytical methods for determination of inorganic arsenic and its metabolites in urine. *Toxicol. Lett.*, 2007, **168**, 310-318.

18 A. Gonzalvez, A. Llorens, M. L. Cervera, S. Armenta and M. de la Guardia, Non-chromatographic speciation of inorganic arsenic in mushrooms by hydride generation atomic fluorescence spectrometry. *Food Chem.*, 2009, **115**, 360-364.

19 H. W. Chen, I. D. Brindle and X. C. Le, Prereduction of arsenic(V) to arsenic(III), enhancement of the signal, and reduction of interferences by L-cysteine in the determination of arsenic by hydride generation. *Anal. Chem.*, 1992, **64**, 667-672

20 J. N. Miller, Basic statistical methods for analytical chemistry Part 2. Calibration and regression methods, *Analyst*, 1991, **116**, 3-14.

21 N. M. M. Coelho, A. C. da Silva and C. M. da Silva, Determination of As(III) and total inorganic arsenic by flow injection hydride generation atomic absorption spectrometry. *Anal. Chim. Acta*, 2002, **460**, 227-233.

22 A. L. Moretto and S. Cadore, Determination of arsenic in food samples by hydride generation - Atomic absorption spectrometry. *Microchim. Acta*, 2004, **146**, 239-244.

23 H. W. Chen, W. Yao, D. X. Wu and I. D. Brindle, Determination of tin in steels by non-dispersive atomic fluorescence spectrometry coupled with flow-injection hydride generation in the presence of L-cysteine. *Spectroc. Acta Pt. B-Atom. Spectr.*, 1996, **51**, 1829-1836.

24 I. D. Brindle and X-C Le, Reduction of interferences in the determination of germanium by hydride generation and atomic emission spectrometry, *Anal. Chim. Acta*, 1990, **229**, 239-247.

25 Y. Maki, K. Kikuchi, H. Sugiyama and S. Seto, Thiol-activated sodium-borohydride reduction of carboxylate ester, *Tetrahedron Lett.*, 1975, **38**, 3295-3296.

26 W. G. Guida, E. E. Entreken and A. R. Guida, Reduction of esters and corboxylates by sodium borohydride/ethahedithiol: improved procedures and an investigation into the nature of the reducing species, *J. Org. Chem.*, 1984, **49**, 3024-3026.

27 A. D'Ulivo, Chemical vapor generation by tetrahydroborate(III) and other borane complexes in aqueous media - A critical discussion of fundamental processes and mechanisms involved in reagent decomposition and hydride formation, *Spectrochim. Acta Part B*, 2004, **59**, 793-825.

28 A. D'Ulivo, J. Dedina, Z. Mester, R. E. Sturgeon, Q. Wang and B. Welz, Mechanisms of chemical generation of volatile hydrides for trace element determination, *Pure Appl. Chem.*, 2011, **38**, 1283-1340.

29 Y. C. Yip, H. S. Chu, C. F. Yuen and W. C. Sham, Determination of inorganic arsenic(III)

and arsenic(V) in water samples by ion chromatography/inductively coupled plasma-mass spectrometry. *J. AOAC Int.*, 2007, **90**, 284-290.

30 T. M. Hsiung and J. M. Wang, Cryogenic trapping with a packed cold finger trap for the determination and speciation of arsenic by flow injection/hydride generation/atomic absorption spectrometry. *J. Anal. At. Spectrom.*, 2004, **19**, 923-928.

31 G. E. Brown, A. L. Foster and J. D. Ostergren, Mineral surfaces and bioavailability of heavy metals: A molecular-scale perspective. *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 3388-3395.

32 P. VanCappellen and Y. F. Wang, Cycling of iron and manganese in surface sediments: A general theory for the coupled transport and reaction of carbon, oxygen, nitrogen, sulfur, iron, and manganese. *Am. J. Sci.*, 1996, **296**, 197-243.

33 L. A. Warren and E. A. Haack, Biogeochemical controls on metal behaviour in freshwater environments. *Earth-Sci. Rev.*, 2001, **54**, 261-320.

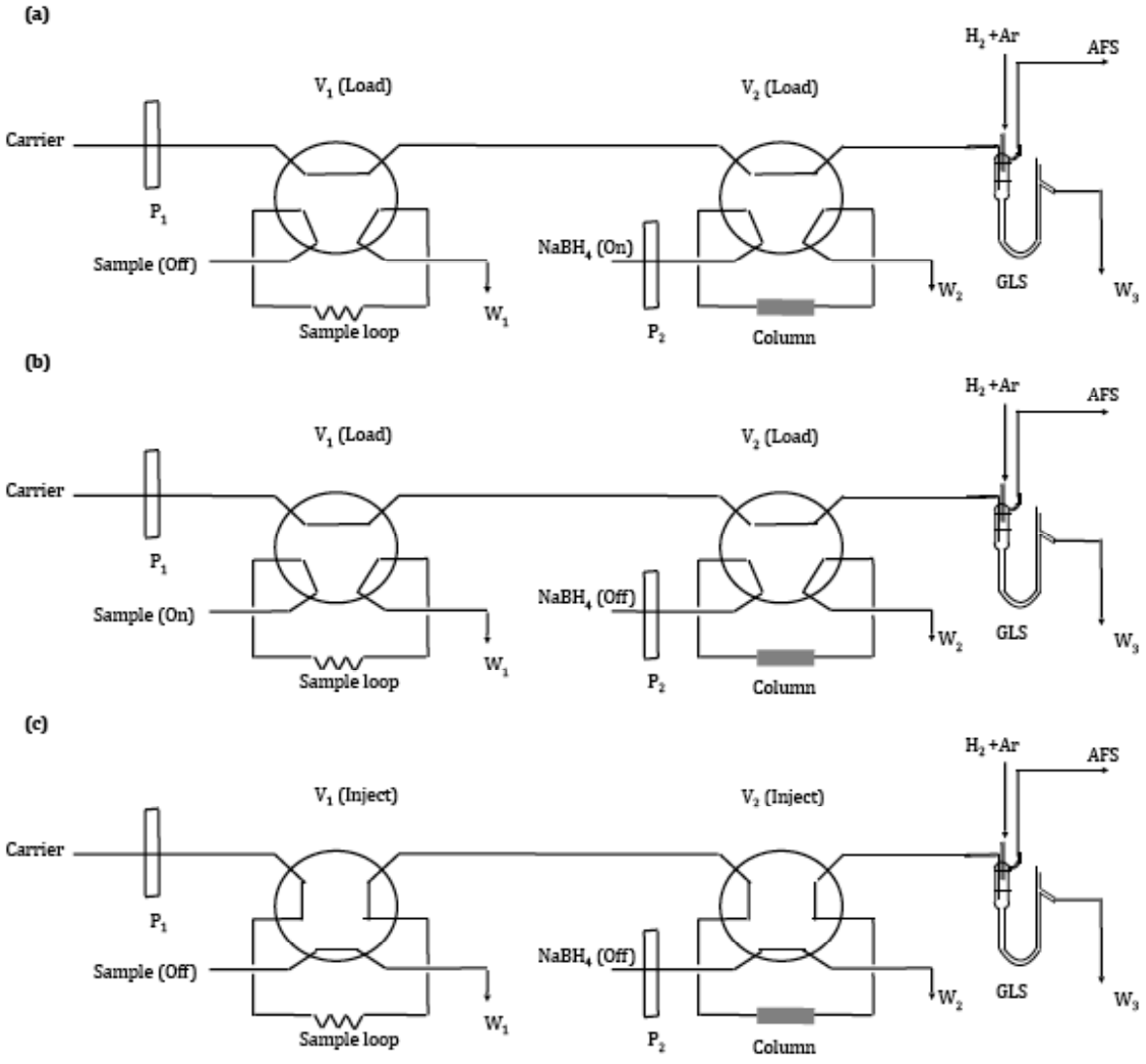

Fig. 1 Manifold for the determination of arsenic by FI-HG-AFS with immobilized tetrahydroborate. V_1, and V_2 are 6-port valve; GLS is the gas–liquid separator; W1, W2, and W3 are waste lines; and P1, and P2 are peristaltic pumps. (a) Both valves are in the load position and borohydride is loaded onto the column mounted in the "loop" of valve V_2. (b) Both valves are in the load position and sample is loaded onto the sample loop of valve V_1 (c) Both valves are in the "inject" position allowing the water carrier to deliver the acidified sample to the borohydride-form anion-exchanger.

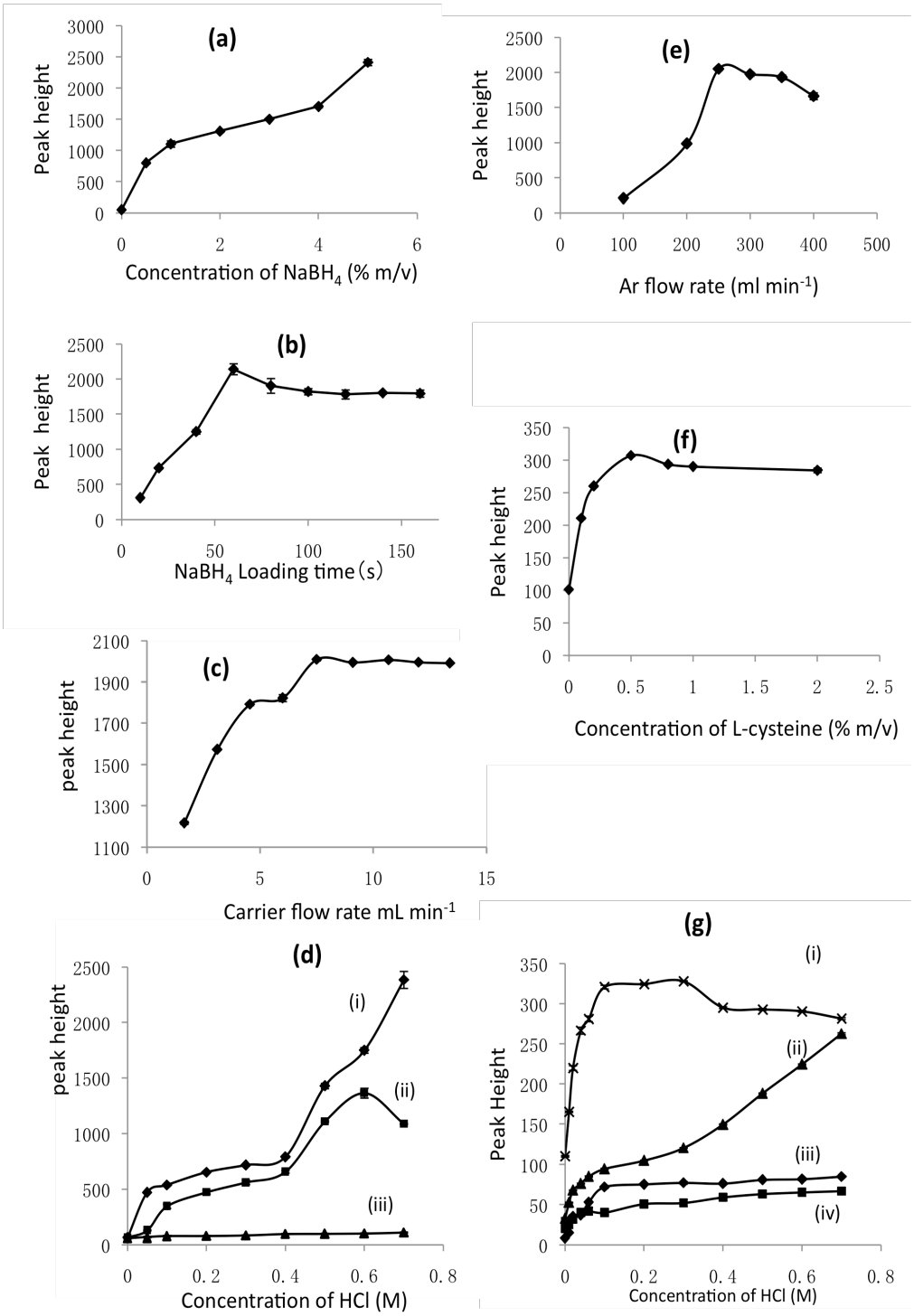

Fig. 2 Effect of (a) $NaBH_4$ concentration, (b) $NaBH_4$ loading time, (c) carrier flow rate, (d) acidity of standard solution (i) 1 µg L^{-1} arsenite, (ii) 1 µg L^{-1} arsenate, (iii) blank], (e) carrier gas flow rate, (f) L-cysteine concentration, and (g) acidity of standard solution with or without the presence of L-cysteine [(i) 1µg L^{-1} arsenite in 0.1M HCl with L-cysteine, (ii) 1 µg L^{-1} arsenite in 0.7 M HCl, (iii) blank of 0.7 M HCl, (iv) blank of 0.1M HCl with L-cysteine. The plots are representative of the effect of each of the individual parameters and were obtained with the values of the other parameters at the optimum values.

Table 1 Parameters and operating conditions of atomic fluorescence spectrometer

Parameter	**Setting**
Primary lamp current	27.5 mA
Boost lamp current	35 mA
Carrier argon flow rate	250 mL min^{-1}
Dryer gas flow rate	2.5 L min^{-1}
Sample flow rate	9.0 mL min^{-1}
$NaBH_4$ flow rate	4.5 mL min^{-1}
H_2 flow rate	80 mL min^{-1}
HCl concentration	
A	0.7 M
B	0.1 M
L-Cysteine concentration	
A	0
B	0.5% (m/v)
$NaBH_4$ concentration	0.7% (m/v)

Table 2 Calibration lines obtained for arsenite and arsenate under conditions A and B

Species	Condition	Equation of fit*	R	LOD (ng L^{-1})	RSD% (0.5 µg L^{-1})
arsenite	**B**	232x + 47	0.99	8	3.8
arsenite	**A**	192x + 61	0.99	13	4.3
arsenate	**B**	233x + 48	0.99	8	3.6
arsenate	**A**	150x + 60	0.99	15	4.1

* x is the concentration in µg L^{-1}

Table 3 Effect of coexisting elements on relative of peak height (%) of 1 μg L^{-1} arsenite.

Element	Concentration (mg L^{-1})	Relative peak height (%)	
		In 0.7 M HCl	In 0.1 M HCl with 0.5%(m/v) L-cysteine
Ca(II)	10	95	99
	100	93	96
Fe(III)	1	68	100
	10	47	98
Mg(II)	10	98	101
	100	107	108
Zn(II)	0.01	95	100
	0.1	93	100
Mn(II)	0.01	100	100
	0.1	108	102
Pb(II)	0.01	100	101
	0.1	99	101
Cu(II)	0.1	89	99
	1	27	97
Se(IV)	0.01	78	86
	1	37	58

Table 4 Inorganic arsenic speciation analysis of fresh water samples.

Sample	Added (μg L^{-1})		Found (μg L^{-1})		Recovery (%)	
	As^{III}	As^{V}	As^{III}	As^{V}	As^{III}	As^{V}
Tap Water	0	0	0.015 ± 0.009*	0.050 ± 0.010		
	0.50	0.50	0.535 ± 0.025	0.524 ± 0.043	104	95
	1.00	1.00	1.11 ± 0.08	1.03 ± 0.09	110	98
Pound Water	0	0	0.026 ± 0.017	0.235 ± 0.034		
	0.50	0.50	0.545 ± 0.035	0.80 ± 0.021	104	113
	1.00	1.00	1.11 ± 0.18	1.28 ± 0.27	108	105
Well Water [1f]	0	0	0.29 ± 0.06	1.05 ± 0.06		
	0.50	0.50	0.75 ± 0.15	1.54 ± 0.20	92	98
	1.00	1.00	1.17 ± 0.25	2.13 ± 0.18	88	108
Well Water [1]	0	0	0.382 ± 0.14	0.85 ± 0.10		
	0.50	0.50	0.95 ± 0.05	1.35 ± 0.24	114	100
	1.00	1.00	1.37 ± 0.28	1.89 ± 0.31	99	104
Well Water [2f]	0	0	0.18 ± 0.09	0.83 ± 0.23		
	0.50	0.50	0.65 ± 0.32	1.32 ± 0.24	94	98
	1.00	1.00	1.16 ± 0.40	1.94 ± 0.36	98	111
Well Water [2]	0	0	0.29 ± 0.21	0.45 ± 0.26		
	0.50	0.50	0.85 ± 0.38	0.93 ± 0.33	112	96
	1.00	1.00	1.39 ± 0.51	1.47 ± 0.45	110	102

[f]First draw in the morning; the other sample was collected after the tap was run for a few minutes

*n = 9, ± terms are 95% confidence intervals

Table 5 Inorganic arsenic speciation analysis of seawater samples.

Sample	Added (μg L^{-1})		Found (μg L^{-1})		Recovery (%)	
	As(III)	As(V)	As(III)	As(V)	As(III)	As(V)
Seawater 1	0	0	0.45 ± 0.03*	0.54 ± 0.01		
	0.50	0.50	0.98 ± 0.08	1.01 ± 0.08	96	94
	1.00	1.00	1.42 ± 0.12	1.50 ± 0.1	97	96
Seawater 2	0	0	0.42 ± 0.08	0.73 ± 0.06		
	0.50	0.50	0.91 ± 0.07	1.20 ± 0.08	98	94
	1.00	1.00	1.47 ± 0.08	1.62 ± 0.05	105	89
NASS-6[#]	0	0	0.26 ± 0.05	1.16 ± 0.07		
	0.50	0.50	0.71 ± 0.16	1.69 ± 0.09	90	106
	1.00	1.00	1.27 ± 0.12	2.11 ± 0.19	101	95

[#]certified value = 1.43 ± 0.12 μg L^{-1}

*n = 9, ± terms are 95% confidence intervals