Design and development of an automated flow injection instrument for the determination of arsenic species in natural waters

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The design and development of an automated flow injection instrument for the determination of arsenite [As(III)] and arsenate [As(V)] in natural waters is described. The instrument incorporates solenoid activated self-priming micropumps and electronic switching valves for controlling the fluidics of the system and a miniature charge-coupled device spectrometer operating in a graphical programming environment. The limits of detection were found to be 0.79 and 0.98 μM for As(III) and As(V), respectively, with linear range of 1–50 μM . Spiked ultrapure water samples were analyzed and recoveries were found to be 97%–101% for As(III) and 95%–99% for As(V), respectively. Future directions in terms of automation, optimization, and field deployment are discussed. © 2009 American Institute of Physics. [doi:10.1063/1.3202083]

I. INTRODUCTION

In natural waters, arsenic exists in a variety of forms including the inorganic species arsenite [As(III)] and arsenate [As(V)] as well as bound to particulates and organic matter.^{1,2} As(III) and As(V) species are considered the most abundant species, with As(V) being thermodynamically stable under oxic conditions.³ In reducing conditions, such as those in naturally occurring geothermal systems,^{3–5} As(III) dominates.⁶ As(III) is more toxic than As(V),⁷ with both affecting the health of millions of people worldwide. Ultimately, the concentrations and speciation of As(III) and As(V) are determined by various abiotic and biologically mediated biogeochemical processes.^{3,8–10}

Traditionally, water quality was assessed by collecting aquatic samples in the field with subsequent measurement of arsenic levels by standard laboratory analytical techniques [e.g., atomic fluorescence spectroscopy, graphite furnace atomic absorption, and inductively coupled plasma-mass spectrometry (ICP-MS)].^{11,12} Such methods excel for routine analysis of total arsenic concentrations but fail in their attempt to measure arsenic speciation [e.g., As(III) and As(V)]. The use of efficient separation techniques (e.g., ion exchange, high performance liquid chromatography, and capillary electrophoresis) coupled with routine detection methods (e.g., ICP-MS) has been shown to allow sensitive and selective determination of arsenic species.^{13–16}

The above laboratory techniques have proved successful in the determination of arsenic in natural waters. Unfortu-

nately, species stability, storage, and preservation are major issues of concern when attempting to maintain the original concentration and reliable speciation information when performing laboratory-based analyses.^{11,17,18} In order to maintain the integrity of the sample and to help prevent possible contamination, sensitive and selective field-based techniques are needed. Various electrochemical,^{19–22} biosensor-based,²³ and colorimetric methods^{24–31} have shown promise for field-based detection of arsenic species.

In this study, a fully automated flow injection-based instrument was designed and developed for the determination of As(III) and As(V) in natural waters. The instrument incorporated self-priming micropumps and electronic switching valves for controlling the fluidics of the system. A graphical user interface was created and used to control automated injection sequences and to allow continuous data acquisition. The instrument incorporated a miniature diode array spectrometer with fiber optic cables for full spectrum acquisition. Laboratory development and analytical performance are presented and future laboratory and field-based studies considered.

II. MATERIALS AND METHODS

A. Reagents and solutions

All solutions were prepared in ultrapure water (Barnstead NanoPURE[®], 18.2 M Ω), and all reagents were of analytical (or equivalent) grade. All containers, bottles and glassware used during this study were cleaned overnight

FIG. 1. Schematic of the prototype FI instrument for the analysis of As(III) and As(V) in natural waters. S=sample, R₁=mixed KI/KIO₃, R₂=ultrapure water, R₃=ammonium molybdate, and R₄=ascorbic acid.

with nutrient free detergent (Neutracon[®], Decon Laboratories, U.K.), rinsed three times with ultrapure water, and soaked in 1% (v/v) HNO₃ for at least 24 h. All were then rinsed three times with ultrapure water and dried at room temperature.

The mixed-iodide/iodate solution was prepared by dissolving 0.3566 g of KI (Sigma) and 0.0834 g of KIO₃ (Mallinckrodt) in 250 ml of ultrapure water. The ammonium molybdate solution was prepared by dissolving 10 g of ammonium molybdate (Fisher) and 28 ml of H₂SO₄ into 1 l of ultrapure water. The ascorbic acid (Fisher) was prepared by dissolving 5.5 g into 100 ml of ultrapure water. As(III) working standards in the range of 0–25 μ M were prepared daily from a stock solution of 500 μ M As₂O₃ (Sigma). As(V) working standards in the range of 0–25 μ M were prepared daily from a stock solution of 500 μ M Na₂HAsO₄·7H₂O (Sigma).

B. Instrument design

A schematic diagram of the completely developed FI system is shown in Fig. 1. The manifold consisted of four solenoid-operated self-priming micropumps (Series 120SP1210-5T, Bio-Chem Valve, NJ, USA) connected to solenoid-operated switching valves (Series 075T3MP12-32, Bio-Chem, NJ, USA) with Teflon tubing (0.80 mm i.d., Omnifit, Cambridge, U.K.). Two operational fluidic format schemes are shown in Figs. 2(a) and 2(b). Switching valves were set to normally open–common (NO/COMM) when de-energized [Fig. 2(a)]. In this operation mode, the sample pump fills the loop (110 μ l) with the sample. The effluent from the loop is captured in a waste container. Switching valves were set to normally closed–common (NC/COMM) when power was supplied to the valves [Fig. 2(b)]. In this mode, the predefined sample amount of 110 μ l is redirected into the carrier stream and mixes with the appropriate reagents for chemical analysis (see description below for complete details). The flow of sample/reagents in

both schemes is shown by darkened lines with arrows. Various reaction coil lengths were tested with this system and the optimum length of 30 cm was used for all reaction coils.

FIG. 2. (a) Fluidic schematic of the FI instrument showing switching valves set to NO/COMM when de-energized. (b) Fluidic schematic of the FI instrument showing switching valves were set to NC/COMM when power was supplied to the valves. The flow of sample/reagents in both schemes is shown by darkened lines with arrows.

TABLE I. 3 day and pooled FI calibration data for As(III).

[As(III)] (μM)	Calibration 1		Calibration 2		Calibration 3		Pooled data	
	Mean absorbance	RSD (%) ($n=3$)	Mean absorbance	RSD (%) ($n=3$)	Mean absorbance	RSD (%) ($n=3$)	Mean absorbance	RSD (%) ($n=3$)
0	0.0002	3.4	0.0002	2.4	0.0001	1.4	0.0001	3.8
2.5	0.0105	3.8	0.0096	5.7	0.0117	3.9	0.0106	9.5
5	0.0249	2.1	0.0239	5.6	0.0239	5.5	0.0242	2.5
10	0.0538	6.0	0.0514	2.9	0.0521	1.8	0.0524	2.3
15	0.0811	1.2	0.0758	4.0	0.0724	3.8	0.0764	5.8
25	0.1177	1.1	0.1141	3.9	0.1118	1.8	0.1145	2.6
r^2	0.9904		0.9937		0.9946		0.9932	
Slope	0.0048		0.0047		0.0045		0.0047	
Intercept (absorbance)	0.0016		0.0001		0.002		0.0015	

A USB4000-UV-Vis miniature fiber optic spectrometer (Ocean Optics, Inc., Dunedin, FL, USA), which includes two 200 μM fiber optic cables featuring a 3648-element linear charge-coupled device array (responsive from 200–850 nm) was used for detection. The light source was a miniature deuterium tungsten halogen lamp (Ocean Optics, Inc., FL, USA). Both were connected to a fiber optic SubMiniature version A (SMA) Z-flow cell (FIALab Instruments, WA, USA) with SMA connectors. An inert polytetrafluoroethylene membrane debubbler (Bio-Chem Valve, NJ, USA) was incorporated to help remove air bubbles from the reagent stream. A five-port injection valve (11500 series, Omnifit, NJ, USA) was incorporated for switching reagents introduced using pump 2. A Topward 3303A dc supply (Test Equity, CA, USA) was used for all power generation.

III. RESULTS AND DISCUSSION

A. Graphical programming environment

A custom graphical program and user interface was written in the LABVIEW (National Instruments Corp., Austin, TX, USA) environment for all manifold control and data acquisition. This development employed a written VIRTUAL INSTRUMENT (VI) in language G with two structures to repeat the execution of a subdiagram: the While Loop and the For Loop. Other structures of G include attribute nodes, arrays, case and sequence structures, and clusters and graphs to display the appropriate data. VIs contain an active user interface (front panel) containing knobs, push buttons, graphs, and other controls and indicators. Within this interface, all pumps, valves, lamp spectrum, spectrometer response, and switching sequences are automatically controlled. In addition, data are collected for each run and sent to a file for storage and further processing.

B. Miniature pump calibration

Linear calibration curves obtained from pumps 2 (carrier), 3 (reagent 1—ammonium molybdate), and 4 (reagent 2—ascorbic acid) were used to convert digital units (0–4) to flow rates (ml min^{-1}). The flow rate for the sample (pump 1) was not important as this only filled the sample loop until injection into the carrier stream. Calibrations of flow rates resulted in good linear correlation with r^2 values of 0.9940,

0.9981, and 0.9923 for pumps 2, 3, and 4, respectively. Based on the calibrations, 0–4 digital units correspond to flow rate ranges of 0–2.3 ml min^{-1} , 0–2.2 ml min^{-1} , and 0–2.5 ml min^{-1} for pumps 2, 3, and 4, respectively.

C. As(III) and As(IV) calibration studies

The molybdenum blue complex method³² routinely used for P(V) detection can also be used for the detection of As(V). Since P(V) will likely interfere with As(V) determination, several modifications of the molybdenum blue method have been reported.^{26,27,31,33,34} The multicomponent determination of phosphorus and arsenic species, including a quantification of the interferences among these species, is beyond the scope of this paper and will be studied at a future date.

For this study, we modified our previously published method³⁴ used for the determination of phosphite P(III) by using a mixed-iodide solution for oxidizing P(III) to P(V). Here, an increased concentration of the mixed-iodide solution (with a subsequent increase in ascorbic acid concentration) was used to oxidize As(III) to As(V) for determination spectrophotometrically at $\lambda_{\text{max}}=700$ nm. Using the mixed-iodide solution, we are able to determine the total arsenic concentration, As_T , in our sample. By switching the five-point injection valve connection to pump 2, the mixed-iodide solution is replaced with ultrapure water. With this carrier flow, only As(V) is determined spectrophotometrically by the molybdenum blue method. The concentration of As(III) is determined as the difference of the concentration of As_T and As(V).

Conditions for the calibration studies were optimized using a univariate approach. Here, one variable (e.g., flow rate) was modified while maintaining the other variables constant. Optimized flow conditions for reagent lines for As(III) were $\text{KI/KIO}_3=0.42$ ml min^{-1} , ammonium molybdate= 0.42 ml min^{-1} , and ascorbic acid= 0.25 ml min^{-1} . Optimized flow conditions for As(V) were as follows: ultrapure water= 0.35 ml min^{-1} , ammonium molybdate= 0.42 ml min^{-1} , and ascorbic acid= 0.25 ml min^{-1} . The flow rate for the sample (for each method) was not important and was used only to fill the sample loop (110 μl) in the injection valve.

TABLE II. 3 day and pooled FI calibration data for As(V).

[As(V)] (μM)	Calibration 1		Calibration 2		Calibration 3		Pooled data	
	Mean absorbance	RSD (%) ($n=3$)	Mean absorbance	RSD (%) ($n=3$)	Mean absorbance	RSD (%) ($n=3$)	Mean absorbance	RSD (%) ($n=3$)
0	0.0001	2.2	0.0001	3.2	0.0001	1.0	0.0001	2.9
5	0.0137	5.1	0.0135	4.6	0.0138	2.5	0.0137	1.1
2.5	0.0278	3.8	0.0279	3.4	0.0283	2.0	0.0280	0.9
10	0.0500	4.5	0.0574	0.8	0.0574	0.9	0.0548	7.5
15	0.0780	3.2	0.0783	3.2	0.0800	1.3	0.0810	3.6
25	0.1323	1.5	0.1341	1.7	0.1360	0.8	0.1341	1.4
r^2	0.9991		0.9985		0.9991		0.9998	
Slope	0.0052		0.0053		0.0054		0.0054	
Intercept (absorbance)	-0.0001		0.0009		0.0008		0.0007	

Comparison of the data for three FI method calibrations for As(III) and As(V) are shown in Tables I and II, respectively, and in Fig. 3. Reproducibility for replicate ($n=3$) injections of As(III) standards (0–25 μM) was typically $<6.0\%$ relative standard deviation (RSD). The pooled data showed good linear correlation ($r^2=0.9932$). For As(V), standard (0–25 μM) reproducibility was typically $<5.0\%$ RSD with good linear correlation ($r^2=0.9998$) for the pooled data. Representative calibration curves for As(III) and As(V) are shown in Figs. 3(a) and 3(b), respectively.

The limits of detection were found to be 0.79 and 0.98 μM for As(III) and As(V), respectively, with linear range of 1–50 μM . For method validation, freshly prepared ultrapure water samples were spiked with 10 μM of As(III) and As(V) and recoveries were measured. Recoveries ranged from 97%–101% for As(III) [mean= $99 \pm 4\%$ ($n=10$)] and 95%–99% for As(V) [mean= $97 \pm 5\%$ ($n=10$)].

The absorbance values at an As(III) concentration of 25 μM is less than the absorbance value at the same concentration of As(V) [see Figs. 3(a) and 3(b)]. This suggests

that the As(III) did not undergo a complete reaction or that the iodide solution interferes with either the molybdenum blue reaction or the detection. This is partly the reason why the linear calibration curve-fit in Fig. 3(a) does not pass through the 15 μM data point. Future work will include an investigation on the effects of the oxidant, the oxidation time, and other environmental parameters on the detection of As(III).

IV. CONCLUSIONS

Here, we present the design and development of a novel instrument capable of measuring arsenic species for high resolution (i.e., analysis every 15 min) monitoring. Because of the variability in the composition of natural waters, it is quite difficult to design a standard storage protocol for collection and preservation of these samples. The proposed sensitive and selective flow-through instrument is field deployable, reducing the need to extract and preserve the water sample. A custom written graphical program and user interface allowed fully automated pumping and switching schemes and data acquisition. Preliminary development and laboratory calibration studies have proved successful with good reproducibility and have been validated by spiked sample studies. The instrument prototype described here is in the early development stages and has been applied successfully in ultrapure water samples. The application of this instrument in field studies is more challenging because of the numerous species (e.g., nitrate and phosphate species) that may interfere with the chemical reactions and the detection. For example, we have previously shown successful oxidation of P(III) to P(V) using the modified molybdenum blue method.³⁴ A similar set of reactions can happen with nitrate species, which are ubiquitous in natural waters.³⁵ By considering the important works of other groups^{19,26,27,30,31} and the methods they used in their arsenic speciation studies and by adding additional pumps and channels that can accommodate additional chemical reaction steps, this instrument should be able to detect a variety of nitrogen, phosphorus, and arsenic species in natural waters.

FIG. 3. Representative calibration curves for the determination of (a) As(V) and (b) As(III) in ultrapure water. The error bars are standard errors for triplicate injections. The solid lines are linear least square fit to the data. The dotted lines represent 95% confidence intervals.

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