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On-line speciation analysis of inorganic arsenic in complex environmental aqueous samples by pervaporation sequential injection analysis

Warunya Boonjob [a,b,c], Manuel Miró [a,*], Spas D. Kolev [c,**]

[a] FI-TRACE group, Department of Chemistry, Faculty of Science, University of Balearic Islands, Palma de Mallorca, Spain
[b] Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[c] School of Chemistry, The University of Melbourne, Victoria 3010, Australia

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ABSTRACT

A proof of concept of a novel pervaporation sequential injection (PSI) analysis method for automatic non-chromatographic speciation analysis of inorganic arsenic in complex aqueous samples is presented. The method is based on hydride generation of arsine followed by its on-line pervaporation-based membrane separation and CCD spectrophotometric detection. The concentrations of arsenite (As(III)) and arsenate (As(V)) are determined sequentially in a single sample zone. The leading section of the sample zone merges with a citric acid/citrate buffer solution (pH 4.5) for the selective reduction of As(III) to arsine while the trailing section of the sample zone merges with hydrochloric acid solution to allow the reduction of both As(III) and As(V) to arsine at pH lower than 1. Virtually identical analytical sensitivity is obtained for both As(III) and As(V) at this high acidity. The flow analyzer also accommodates in-line pH detector for monitoring of the acidity throughout the sample zone prior to hydride generation.

Under optimal conditions the proposed PSI method is characterized by a limit of detection, linear calibration range and repeatability for As(III) of 22 μg L^{-1} ($3s_{blank}$ level criterion), 50–1000 μg L^{-1} and 3.0% at the 500 μg L^{-1} level and for As(V) of 51 μg L^{-1}, 100–2000 μg L^{-1} and 2.6% at the 500 μg L^{-1} level, respectively. The method was validated with mixed As(III)/As(V) standard aqueous solutions and successfully applied to the determination of As(III) and As(V) in river water samples with elevated content of dissolved organic carbon and suspended particulate matter with no prior sample pretreatment. Excellent relative recoveries ranging from 98% to 104% were obtained for both As(III) and As(V).

1. Introduction

Inorganic arsenic, including arsenite (As(III)) and arsenate (As(V)), dominates in surface and ground waters with concentrations of various orders of magnitude greater than those of organic arsenic compounds. The toxicity of arsenic strongly depends on its chemical forms, with arsenite being the most hazardous inorganic arsenic species in the food webs [1–3]. For this reason arsenic speciation is required for reliable assessment of potential deleterious effects of waterborne arsenic on biota. A wealth of analytical methods for arsenic speciation have been reported over the past decade [2–5] involving (i) spectrophotometric detection of the heteropolymolybdoarsenic acid or indirect optical methods [6,7], (ii) hydride generation atomic spectrometric detection [8–10], (iii) electrochemical detection based on stripping analysis or amperometry [11,12], and (iv) hyphenated chromatographic techniques with mass spectrometric detection [3,13–17]. In addition to utilizing expensive instrumentation and consumables, atomic and mass spectrometric techniques are inappropriate for field measurements because of the corresponding bulky equipment and the large amounts of gases or mobile phases required [18]. Flow analysis manifolds furnished with portable optical detectors are deemed to be affordable alternatives to the above mentioned spectrometric techniques which also offer simplification and miniaturization of arsenic speciation assays [18–22]. Isolation of hydride species in flow analyzers is usually accomplished by either gas-diffusion or pervaporation-based membrane separation. Analytical pervaporation is the combination of evaporation and gas-diffusion in a single module [23–25]. Volatile analytes evaporate first in the headspace of the donor chamber of the pervaporation cell before diffusing across a hydrophobic porous membrane into the acceptor solution located in the acceptor chamber. Pervaporation is more appealing than gas-diffusion alone inasmuch as the sample matrix is not in contact with the membrane and affords improved membrane reusability with minimal fouling risks [23,26]. The pervaporation flow injection (PFI)

* Corresponding author. Tel: +34 971172746; fax: +34 971173426.
** Corresponding author. Tel.: +61 3 83447931; fax: +61 3 9347 5180.
E-mail addresses: manuel.miro@uib.es (M. Miró), s.kolev@unimelb.edu.au (S.D. Kolev).

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Table 1
Physicochemical characterization of the river water samples analyzed in this work.

Sample number	Na (mg L^{-1})	Ca (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Zn (mg L^{-1})	Pb (mg L^{-1})	Ni (mg L^{-1})	pH	Turbidity (NTU)	Conductivity (mS)	Suspended solids (mg L^{-1})	Dissolved organic matter (mg L^{-1})
1	5000	147	240	178	0.56	0.04	0.01	6.85	30	33	263	7.6
2	4500	134	224	176	0.31	0.02	ND[a]	6.56	30	34	250	7.0
3	5100	150	248	180	0.30	0.04	ND[a]	6.42	17	47	257	6.5

[a] Not detected.

methods for the determination of inorganic arsenic involve hydride generation of arsine gas from As(III) and As(V) in the donor stream with subsequent arsine detection in the acceptor stream by methods such as spectrophotometry (e.g. bleaching of $KMnO_4$ [7] or oxidation of arsine to arsenate followed by the molybdenum blue reaction [27]), amperometry [12] or atomic fluorescence spectrometry [28]. These methods [7,12,23,27] lack automation because of manual injection of samples and standards and are inappropriate for on-line speciation analysis whenever L-cysteine [12] or a mixture of KI and HCl [7,27] are employed as reducing reagents because of slow reduction kinetics. In some instances, there was the need of applying higher temperature (e.g. 70 °C [7]) to increase the rate of the derivatization reaction in the acceptor stream (e.g. molybdenum blue method) with the consequent generation of nuisance bubbles. However, the most serious limitation of these methods, which is arguably valid for all PFI methods, is the difficulty of maintaining a constant headspace volume in the donor chamber to obtain reproducible results. This usually requires the use of two precisely synchronized peristaltic pumps, one for delivering the donor solution into the donor chamber and another one for aspirating it at the same flow rate [7,12,27,29–31].

To tackle the above drawbacks, a fully automatic sequential injection analysis (SIA) manifold, equipped with stand-alone high precision syringe pumps in a hybrid flow configuration, is proposed as a novel flow analysis platform for conducting on-line analytical pervaporation. It is expected that this flow configuration will improve the performance characteristics and provide ease of operation of analytical pervaporation by exploiting flow programming for precisely metering of sample and reagent volumes and controlling flow rates. To the best of our knowledge, the combination of analytical pervaporation with SIA has not been reported in the literature as of yet. A proof of concept of coupling of SIA with analytical pervaporation separation in a novel pervaporation sequential injection (PSI) based hybrid flow configuration is presented in this work. This manifold is used for automatic non-chromatographic speciation of waterborne inorganic arsenic in a single sample plug by varying the pH at which arsine is generated. Concomitant on-line elimination of interferences caused by high turbidity and elevated content of organic matter in the sample is achieved as a result of the pervaporation separation of arsine from the sample matrix prior to detection.

2. Experimental

2.1. Reagents

All chemicals were of analytical reagent grade. Deionised water (18.2 MΩ cm, Millipore, France) was used in the preparation of all solutions. Stock solutions (1000 mg L^{-1}) of As(III) and As(V) were prepared by dissolving 0.1720 g of $NaAsO_2$ (Ajax, Australia) and 0.4182 g Na_2HAsO_4 (Ajax, Australia), respectively, in 1 mL of 5.0 mol L^{-1} NaOH (Merck, Germany), each solution was neutralized with 1.0 mol L^{-1} H_2SO_4 (Ajax, Australia) using phenolphthalein (Ajax, Australia) as the indicator and diluted to 100 mL with deionised water. Working standard solutions of As(III) and As(V) were prepared daily by stepwise dilution at the desired concentrations in 0.3 mol L^{-1} HCl (BDH, Australia). Potassium permanganate stock solution (1.00×10^{-2} mol L^{-1}) was prepared by dissolving 0.1580 g of $KMnO_4$ (BDH, Australia) in 100 mL of 0.2 mol L^{-1} H_2SO_4. The acceptor solution consisted of 3.00×10^{-4} mol L^{-1} $KMnO_4$ in 0.2 mol L^{-1} H_2SO_4. A 5.0 mol L^{-1} HCl solution was prepared by diluting 49.27 mL of concentrated HCl (37% HCl, BDH, Australia) to 100 mL with deionised water. A 0.2 mol L^{-1} sodium borohydride solution (BDH, Australia) was prepared fresh daily by dissolving 0.76 g of $NaBH_4$ in 100 mL of 0.05 mol L^{-1} NaOH. A buffer solution containing 1.0 mol L^{-1} citric acid/citrate was prepared by dissolving 21.14 g of citric acid (BDG, Australia) in 1000 mL of deionised water to which 5 mol L^{-1} NaOH was added until pH 4.5 was reached.

2.2. Samples collection and characterization

Three samples collected along the watershed of Marybyrnong River (Melbourne, Australia) were analyzed for inorganic arsenic in the PSI manifold and the method trueness was checked by spike recovery experiments. The samples were characterized in terms of their turbidity, electric conductivity, pH, suspended solids, major and trace elements and dissolved organic carbon (Table 1). Turbidity and conductivity measurements were undertaken with handheld meters (ICM, Hillboro, USA). Concentrations of selected metals were determined by an inductively coupled plasma optical emission spectrometer (ICP-OES) equipped with a cross-flow nebulizer (Vista-MPX, Varian) following manufacturer's recommendations. Samples were filtered through 0.45 μm Nylon filters for determination of the percentage of suspended particulate matter. The filtered and acidified water samples were then sparged with oxygen to remove inorganic carbon for determination of dissolved organic carbon by non-dispersive infrared detection after combustion at > 600 °C.

2.3. PSI manifold for on-line arsenic speciation

An FIAlab-3200 (FIAlab, USA) SIA system equipped with an internally incorporated 8-port multiport selection valve (SV) and two bi-directional syringe pumps (Cavro, USA) was employed as the flow analysis platform (Fig. 1). The two syringe pumps (SP) labeled as SP1 and SP2 with a capacity of 2.5 and 1.0 mL, respectively, were used for propelling the carrier (deionised water) and acceptor solution (3.0×10^{-4} mol L^{-1} $KMnO_4$ in 0.2 mol L^{-1} H_2SO_4), respectively. SP1, connected to the SV via the 3.5 mL holding coil (HC) made of polytetrafluoroethylene tubing (PTFE, 0.8 mm ID), was employed for automatic handling of precisely metered volumes of standards and samples. Three external stand-alone syringe pumps (microCPS-300, FIAlab, USA) labeled as SP3, SP4 and SP5 (Fig. 1) with a capacity of 2.5 mL each were used for propelling the citric acid/citrate buffer, hydrochloric acid and sodium borohydride solutions, respectively. All syringe pumps were independently programmable. SV incorporated a central port and a communication channel (CC) that could be programmed to link the central port to any of the peripheral ports which were

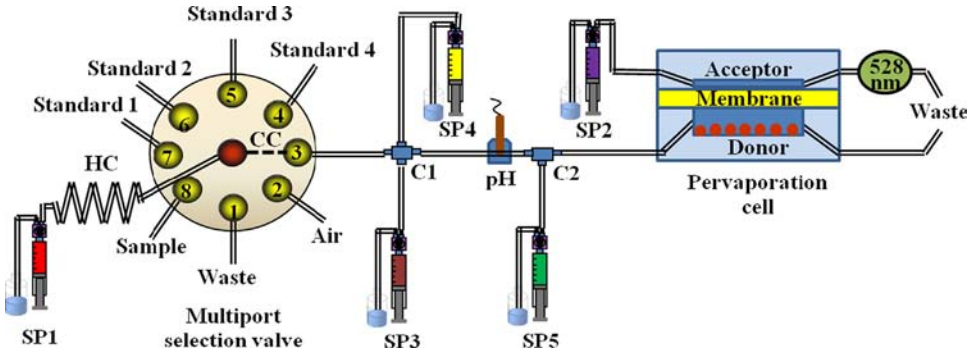

Fig. 1. Schematic of the experimental PSI manifold (SP – syringe pump, SP1 – deionised water, SP2 – $KMnO_4$, SP3 – 0.1 M citric acid/citrate buffer, SP4 – 5 M HCl, SP5 – 1 M $NaBH_4$, ● – glass beads, CC – communication channel, HC – holding coil).

connected to various fluids (e.g. sample, standards and air). The manifold also contained triple and quadruple PTFE connectors (C_1 and C_2) for merging of solution streams.

The pervaporation cell used in this work is described elsewhere [30]. It consisted of two circular Perspex blocks (61 mm diameter, 25 mm high) held together by stainless steel ring clamps and four stainless steel bolts. Both the acceptor (0.3 mm deep) and donor (5.0 mm deep) chambers were hexagonal in shape and a single layer of glass-beads (3.0 mm diameter, Selby-Biolab, Australia) was placed on the bottom of the donor chamber for improved mixing of the borohydride solution with the sample zone. A circular semi-permeable PTFE membrane (1.5 mm thick; pore size: 2 μm; diameter: 40 mm, Trace Biotech, Germany) was sandwiched between the donor and acceptor chambers of the pervaporation cell. The PSI manifold (Fig. 1) was fully controlled by the FIAlab software run under Windows XP.

A fiber optic CCD spectrometer (USB2000-UV–vis, Ocean Optics, USA) was used for measurement of the bleaching of the dilute permanganate acceptor stream as a result of the oxidation of arsine. A Ultem SMA Z-flow cell of 10 mm optical path length was connected to the spectrometer and to a tungsten halogen lamp using optical fiber cables (P600-20, 600 μm quartz core, 20 in. long, SMA connectors on each end, FIAlab, USA). Detection parameters and data acquisition were controlled using the FIAlab software.

In-line pH measurement of the donor stream was undertaken in real time as a quality control tool using a flow-through pH microelectrode and pH portable meter (Jenco Electronic, Ltd, San Diego, USA). The flow-through electrode body made from acrylic has an internal volume of 50 μL and the electrode showcases less than 20 s response time for a 50% change in pH. In-line pH data were recorded by the Arrow pH TM Plotter software.

2.4. Arsenic speciation in the experimental PSI manifold

2.4.1. Approach

The analytical procedure involves in-line evolvement of arsine into the headspace of the donor chamber from either As(III) or total inorganic arsenic (As(III)+As(V)) followed by its transfer into the permanganate oxidant acceptor stream across the hydrophobic semi-permeable porous membrane. The bleaching of permanganate is monitored at 528 nm using a reference wavelength of 715 nm. The PSI method involves automatic sequential determination of As(III) and total inorganic arsenic in a single 2-mL sample zone by manipulating the acidity at which arsine is generated. The first milliliter of sample in the holding coil (HC, Fig. 1) is used to selectively determine As(III) at pH 4.5 using 1 mol L^{-1} citric acid/citrate buffer as an on-line pH adjustment reagent. pH is monitored in-line using the pH microelectrode described above. Arsenate (As(V)) does not generate arsine at this pH. The second milliliter of sample merges downstream with a metered volume of 5 mol L^{-1} HCl so that the acquired final acidity of 1.7 mol L^{-1} of the donor stream ensures that both arsenite and arsenate are quantitatively reduced to arsine. On account of the difference in the headspace volume at pH 4.5 (detection of As(III)) and 1.7 mol L^{-1} HCl (detection of both As(III) and As(V)) caused by the varying amounts of H_2 generated, the rate of arsine transfer into the acceptor solution depends upon the reaction medium acidity. This results in different sensitivity for As(III) and total As (As(III)+As(V)) and therefore two calibration curves (Eqs. (1) and (2)) are required for arsenic speciation. The concentrations of As(III) and As(V) are calculated by simultaneously solving these two equations.

$$S_{(\mathrm{pH}=4.5)} = a_{(\mathrm{pH}=4.5)}[\mathrm{As(III)}] + b_{(\mathrm{pH}=4.5)} \quad (1)$$

$$S_{([\mathrm{HCl}]=1.7\mathrm{M})} = a_{([\mathrm{HCl}]=1.7\ \mathrm{M})}([\mathrm{As(III)}]+[\mathrm{As}(V)]) + b_{([\mathrm{HCl}]=1.7\ \mathrm{M})} \quad (2)$$

The suitability of both the absolute value of the maximum absorbance decrease relative to the baseline at 528 nm (peak height) and the absolute area of the absorbance peak (peak area) as analytical signal (S) can be considered. Peak area is calculated by PeakFit software using the original peak data collected by FIAlab software. Preliminary experiments involving 12 replicate injections have revealed that peak height measurements provide significantly better repeatability than peak area measurements and therefore the absolute value of peak height has been used as the analytical signal (S) in the present study.

2.4.2. Analytical procedure

The stopped flow approach is used in the proposed manifold for sensitivity improvement. Initially, SP2 is activated to pump a metered volume of 250 μL of a solution containing 3.00×10^{-4} mol L^{-1} $KMnO_4$ and 0.2 mol L^{-1} H_2SO_4 at a flow rate of 1.5 mL min^{-1} towards the acceptor chamber of the pervaporation cell having a dead volume of ca. 120 μL to completely fill it whereupon the flow is stopped (Fig. 1). Next, SP1 aspirates consecutively into HC (Fig. 1) 20 μL of air (Port 2), 2 mL of sample (Port 8) and 20 μL of air (Port 2) at 6 mL min^{-1}. The flow direction is reversed and sample plug is pumped towards the pervaporation cell at a flow rate of 0.15 mL min^{-1}. When the front edge of the sample plug reaches connector C1, SP3 starts pumping citric acid/citrate buffer at a flow rate of 0.15 mL min^{-1} to ensure the merging of the first milliliter of the sample with the citric acid/citrate buffer at a 1:1 ratio. When the front edge of the combined sample/buffer zone reaches connector C2, SP5 is programmed to start pumping the sodium borohydride solution at a flow rate of 0.15 mL min^{-1} thus bringing the total donor stream flow rate to 0.45 mL min^{-1}. Arsine generated as a result of the reaction between As(III) and BH_4^- diffuses through the headspace and the membrane of the pervaporation cell into the static acidic permanganate acceptor solution. Arsine is oxidized in the acceptor chamber thus decreasing the concentration of permanganate accordingly. Once SP3 and

SP5 have delivered 1 mL of buffer and 1 mL $NaBH_4$ solutions, respectively, they deliver an additional 180 μL of buffer solution (SP3, flow rate of 0.30 mL min^{-1}) and 50 μL of $NaBH_4$ solution (SP5, flow rate of 0.15 mL min^{-1}) to transport the remaining of the first milliliter of the sample in the tubing connecting C2 and the pervaporation cell to the donor chamber of the pervaporation cell. After this fluidic operation has been completed all syringe pumps remain stopped for 60 s to allow the arsine in the headspace of the donor chamber to diffuse across the membrane into the stagnant receiving solution in the acceptor chamber. After this delay time the stagnant acceptor solution is propelled by SP2 at a flow rate of 1.2 mL min^{-1} towards the spectrophotometric flow-through measuring cell where the absorbance of the permanganate acceptor solution is monitored continuously at 528 nm by the CCD detector.

After recording the readout for As(III) at pH 4.5, the second milliliter of the 2 mL sample stored in the HC is processed in a similar manner as the first milliliter with the only difference being that instead of delivering buffer solution by SP3, 5 mol L^{-1} HCl solution is delivered by SP4.

A cleaning step is required prior to analyzing another sample and it consists of washing the donor and acceptor channels with 2 mL of deionised water pumped by SP1 at a flow rate of 3.0 mL min^{-1} and 1 mL of permanganate solution pumped by SP2 at a flow rate of 1.5 mL min^{-1}, respectively.

The total analysis time of a sample, including the cleaning step, is 19.4 min which corresponds to 9.7 min for each of the two measurements (i.e. As(III) and total As) per sample.

2.4.3. Two-level factorial design

A well-defined number of experiments according to a two-level full-factorial experimental design was conducted to ascertain whether the concentrations of borohydride and hydrochloric acid and the total donor stream flow rate (sum of the sample, $NaBH_4$, HCl or citric acid/citrate buffer flow rates) had a significant influence on permanganate bleaching in the acceptor stream. The relevance of the interaction between these three factors was evaluated as well. The analysis of response data was performed in a dimensionless coordinate system using factor coding. In this factor space, the highest, center point and lowest levels are denoted as +1, 0 and −1, respectively. The experimental domain (uncoded values) for $NaBH_4$, HCl and total flow rate were determined as 0.2–1.0 mol L^{-1}, 1.0–5.0 mol L^{-1} and 0.45–1.35 mL min^{-1}, respectively, on the basis of preliminary experiments done in univariate format. Three replicates of the center point were also included in the design to ensure that the variability found was caused by the factor studied rather than by random errors. Notwithstanding the fact that the replicate measurements were only conducted for the center point, the calculated uncertainty was utilized as an estimate of the variability for the entire experimental domain.

The statistical computer package StatGraphics (Stat-Graphics Centurion XV, Stat Point, Herndon, VA, USA, 2005) was used to build the two-level factorial design (3 parameters) with a total number of eleven runs, including the center points. The effects of the individual factors and their second order interactions were thus investigated and presented in a Pareto chart (see below).

3. Results and discussion

3.1. Optimization of the PSI manifold

The optimization of the PSI manifold was based on a full factorial design at two levels with three replicates of the center point (see Section 2). The concentrations of borohydride, hydrochloric acid and the total donor stream flow rate (sum of the sample, $NaBH_4$, HCl or citric acid/citrate buffer flow rates) were selected as the potentially relevant factors. The concentration of citric acid/citrate buffer was fixed at 0.10 mol L^{-1} (pH 4.5) in accordance with the findings of Idowu et al. [18].

The significance of the influence of the main factors and their interactions upon analytical readouts was evaluated using ANOVA. The standardized factor effects are shown in a Pareto chart (Fig. 2) in descending order [32]. Each bar length equates the value of a calculated Student's *t*. A given factor effect is deemed statistically significant whenever its *t*-value is equal or larger than the critical *t* value at the 0.05 significance level. In the case of As (V) determination the critical *t* value for 4 degrees of freedom at the 0.05 significance level is equal to 2.77 (represented by the vertical line in Fig. 2).

The Pareto chart results for As(V) (Fig. 2) revealed that the concentrations of borohydride and hydrochloric acid, and the total donor flow rate were statistically significant within the experimental domain at the 0.05 significance level. Analytical readouts for As(V) increased at higher acidity and lower flow rate of the donor stream. Lowering the flow rate increased the sample residence time in the donor chamber of the pervaporation cell thus providing longer time for reduction of As(V) to arsine and its subsequent diffusion through the headspace and across the membrane into the acceptor solution. Better sensitivity was observed at lower concentrations of borohydride. It should be borne in mind that the increase in the reducing reagent concentration jeopardizes the analytical performance because of undue generation of hydrogen as a result of which high blank signals and noisy baselines were recorded.

As for the determination of As(III), the effect of all three experimental parameters studied was deemed as insignificant at the 0.05 significance level within the investigated experimental domain. More importantly, none of the two-factor interactions for either As(III) or As(V) were proven statistically significant. Therefore, it was concluded that univariate rather multivariate optimization methods could be used for these three parameters without affording biased results.

Two pumps are usually required in PFI manifolds for controlling the liquid level in the donor chamber of the pervaporation cell [7,12,27,29,30]. In the proposed PSI manifold, the use of a single pump was found to be sufficient for maintaining a constant liquid level in the donor chamber regardless of the donor flow rate of up to 6.0 mL min^{-1} provided that the outlet of the donor chamber was connected to a large bore tubing (2.54 mm ID) for minimum pressure drop and efficient withdrawal of evolved hydrogen in the donor chamber.

Table 2 summarizes the optimal values of the main system parameters and the working range within which each parameter was studied.

3.2. Analytical figures of merit

The analytical figures of merit of the proposed automated PSI method for on-line As speciation were determined under optimal

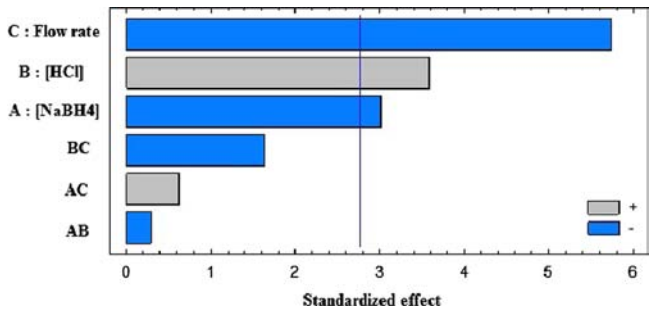

Fig. 2. Pareto chart of the standardized effects (0.05 significance level) for the on-line generation of arsine from As(V) in the experimental PSI manifold.

Table 2
Optimization of the PSI manifold parameters.

Manifold parameter	Range studied	Optimal value
Donor half		
$NaBH_4$ concentration in 0.05 mol L^{-1} NaOH (mol L^{-1})	0.2–1.0	0.2
Nominal HCl concentration (mol L^{-1})	0.3–5.0	5.0
Total donor flow rate (mL min^{-1}) (sample+citric acid buffer or HCl+$NaBH_4$)	0.45–1.35	0.45
Acceptor half		
$KMnO_4$ concentration in 0.2 mol L^{-1} H_2SO_4 (mol L^{-1})	1.0×10^{-4}–5.0×10^{-4}	3.0×10^{-4}
Delay time (s)	30–120	60

Table 3
Relative recoveries of spiked with As(III) and As(V) deionized water standards and river water samples.

Sample	Spiked (μg L^{-1})		As(III)/As(V) ratio	Found (μg L^{-1})		Recovery (%)	
	As(III)	As(V)		As(III)	As(V)	As(III)	As(V)
Synthetic sample	100	400	1.0:4.0	112 ± 11	391 ± 11	112 ± 11	98 ± 3
Synthetic sample	400	100	4.0:1.0	406 ± 7	100 ± 11	101 ± 2	100 ± 11
Synthetic sample	500	500	1.0:1.0	506 ± 15	503 ± 31	101 ± 3	101 ± 6
River water no. 1	150	350	1.0:2.3	147 ± 7	349 ± 7	98 ± 5	99 ± 3
River water no. 2	350	150	2.3:1.0	359 ± 11	156 ± 24	102 ± 3	104 ± 16
River water no. 3	250	250	1.0:1.0	254 ± 5	252 ± 16	102 ± 2	101 ± 6

experimental conditions (Table 2). The method is characterized by linear calibration ranges for As(III) of 50–1000 μg L^{-1} ($S=1.26\times10^{-4}$ [As(III)]+2.60×10^{-3}, $R^2=0.999$) and total As of 100–2000 μg L^{-1} ($S=7.51\times10^{-5}\times$([As(III)]+[As(V)])+$2.25\times10^{-3}$, $R^2=0.999$), respectively. It should be pointed out that under the acidic conditions for total As determination (1.7 mol L^{-1} HCl) the same sensitivity was observed for both As(III) and As(V) thus suggesting that both As species were quantitatively reduced to arsine. As discussed earlier, at the higher acidity for total As determination a substantially higher rate of H_2 generation was observed which resulted in a larger volume of headspace compared to the headspace volume at pH 4.5 (As(III) determination). Therefore, the rate of transfer of arsine to the acceptor solution in the case of total As determination (i.e. higher acidity condition) was lower than that in the case of As(III) only determination (i.e. lower acidity conditions), which explains the slightly different slope of the two calibration curves and the lower limit of detection (LOD) for total As (51 μg L^{-1}) compared to that of As(III) only (22 μg L^{-1}). The LODs were calculated according to the $3s_{blank}$ level criterion.

To the best of our knowledge this is the first PSI method for arsenic speciation and therefore its sensitivity and sampling rate are compared to those of PFI methods for As [7,12,23,27]. These methods have been used for the determination of either As(III) or total inorganic As and in some cases off-line As speciation has been conducted. The LOD of the proposed PSI method (22 μg L^{-1} for As(III)) is slightly outside the upper range of the LODs of the PFI methods mentioned above (i.e. 0.14–14 μg L^{-1}). The lower sensitivity of the proposed PSI method can be attributed to differences in the flow configuration and experimental conditions. For example, the acceptor stream in the PFI system, developed earlier by us [27] and utilizing the same detection chemistry, is stopped for 6.5 min after sample loading in contrast to merely 60 s in the proposed PSI system. Moreover, the sample zone subjected to speciation analysis in the proposed PSI system merges with either a buffer solution or concentrated HCl solution and therefore the sample entering the pervaporation unit is more diluted than the one in the PFI system mentioned above. The sampling rate of 9.7 min per arsenic species (As(III) or As(V)) is within the range of sampling rates (5–15 min) offered by the PFI methods mentioned above [7,12,23,27]. However, unlike these PFI methods, the proposed PSI method offers fully automatic on-line speciation capabilities.

Precision is calculated as the relative standard deviation (RSD) of ten consecutive injections of a mixed standard of As(III) and As(V) at the 500 μg L^{-1} level. RSD of 3.0% and 2.6% were found for As(III) and As(V), respectively. The reuse of a single membrane for more than 500 injections was proven feasible on account of the absence of membrane fouling or water condensation since the manifold operates at room temperature.

3.3. Interference studies

The optimized PSI method was shown to be immune to interferences of commonly found anions in environmental waters with tolerance of chloride, carbonate, nitrate, orthophosphate and sulfate at concentrations of up to 1000 mg L^{-1} at the 10% interference level for As(III) and As(V) concentrations of 500 μg L^{-1}. As expected, sulfide, which in acidic medium produces the reducing gas H_2S, was found to exhibit significant positive interference at concentrations similar to those of As(III) and As(V). However, this interference was overcome by prior sample acidification to pH < 1 and sparging with nitrogen for removal of hydrogen sulfide which was followed by sample neutralization before analysis. Alternatively, lead nitrate at the 100 mg L^{-1} level could be added to the sample for precipitation of lead sulfide followed by speciation analysis of the supernatant in the PSI manifold. Hydride and cold vapor forming metal/metalloid species such as Se(IV), Sn(II), Sb(III), and Hg(II) were tolerated to the same concentration level as As(III) and As(V) at the 10% interference level as a result of kinetic discrimination in the on-line reduction of these metal/metalloid species and/or the lower rate of diffusion of the corresponding volatile species across the headspace and the membrane in the pervaporation cell. The tolerance ratio for Se(IV) and Hg(II) to As was increased up to 50 by adding 4 g L^{-1} hydrazinium sulfate and 0.2 g L^{-1} L-cysteine to the sample, respectively.

3.4. Validation of the PSI speciation method

Investigation of the speciation capabilities of the PSI manifold for discrimination of As(III) and As(V) at varying concentration levels was performed by using deionised water standards with As (III)/As(V) concentration ratios spanning from 0.25 to 4 (Table 3).

Relative recoveries within the range 98–112% were obtained for the suite of assayed ratios.

For investigation of method trueness, the PSI method was applied directly (no off-line sample pretreatment) to spiked with As(III) and As(V) complex environmental aqueous samples from Marybyrnong River (Victoria, Australia) characterized by high turbidity and organic matter content (Table 1). The concentrations of As(III) and As(V) in the original samples were below the LOD of the proposed PSI method. External calibration was utilized for calculation of the relative recoveries of spiked samples at varying concentration levels of As(III) and As(V). Relative recoveries within the range of 98–104% were obtained for both As(III) and As(V) in all spiked samples analyzed (Table 3). The *t*-test indicated that there was no statistically significant difference between added and determined concentration for both As(III) and As(V) at the 0.05 significance level. Hence, it was concluded that despite the complexity of the river samples the PSI method was free from multiplicative matrix interferences and therefore there was no need for applying the standard additions method.

4. Conclusions

This paper presents the first, to the best of our knowledge, proof of concept of the hyphenation of SIA and on-line analytical pervaporation separation for the determination of volatile or semi-volatile analytes or analytes that can be converted chemically to volatile species in complex aqueous samples (e.g. matrices containing macromolecules and fine suspended particulate matter). The proposed hyphenated technique combines the benefits of advanced programmable flow manipulation with non-chromatographic on-line membrane separation. These advantages are illustrated with the development and subsequent application of a spectrophotometric PSI method for inorganic arsenic speciation in 'dirty' river water samples without off-line sample pretreatment based on hydride generation. Due to the mesofluidic manipulation capabilities of SIA it was possible to adjust the sample zone acidity at its leading and trailing sections in an on-line fashion to allow selective determination of arsenite and total inorganic arsenic as the sum of arsenite and arsenate. The concentration of arsenate is calculated as the difference between the concentrations of total arsenic and arsenite. Due to the simple and inexpensive detection approach used which is based on the reduction of permanganate in the acceptor stream by arsine and permanganate monitoring by CCD spectrophotometry, the sensitivity of the proposed method is comparable or lower to that of pervaporation methods employing the flow injection principle. However, this is heavily outweighed by the on-line speciation capabilities of the proposed PSI technique, automatic flow programming and its economy with respect to reagent consumption in line with green chemical principles. In addition, the proposed manifold offers improved reliability and ruggedness compared to PFI manifolds as a single pump is required to control and maintain precisely the liquid level in the donor chamber, which is a major issue under flow injection conditions. Furthermore, the PSI manifold requires minimal operational maintenance inasmuch as a single hydrophobic membrane can be reused for more than 500 injections regardless of the sample complexity. All these advantageous features of the proposed hyphenated technique, illustrated with the successfully conducted recovery experiments outlined in the present paper, are expected to promote its future analytical applications.

Current work is underway on implementing the proposed PSI technique to on-line dynamic chemical fractionation of inorganic arsenic in environmental solids for evaluation of bioaccessible arsenic species under worst-case scenarios.

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