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Miniaturised isotachophoretic analysis of inorganic arsenic speciation using a planar polymer chip with integrated conductivity detection

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Abstract

A new method allowing the analysis of inorganic arsenic species using isotachophoresis has been developed. This method has been shown to be suitable for use on both miniaturised planar polymer separation devices and capillary scale devices. A poly(methyl methacrylate) chip with integrated conductivity electrodes has been successfully used for the rapid analysis of inorganic arsenic species in under 600 s. Limits of detection of 1.8 mg l^{-1} and 4.8 mg l^{-1} for arsenic(V) and arsenic(III), respectively, have been achieved with the miniaturised device. The device has also been used to perform the simultaneous separation of arsenic(III), arsenic(V), antimony(III), molybdenum(VI) and tellurium(IV).

Keywords: Isotachophoresis; Chip technology; Instrumentation; Conductivity detection; Detection, electrophoresis; Inorganic anions; Arsenic

1. Introduction

Arsenic species are widely found in the environment, being present in the atmosphere, sea water, freshwater and in the soil. Arsenic species are known to be highly toxic. Human exposure can result in acute or chronic poisoning and there is sufficient evidence for the carcinogenicity of arsenic compounds in humans [1]. Therefore the levels of arsenic permitted in industrial wastewater and drinking water are highly regulated. For example, a limit of 50 μg l^{-1} of arsenic in drinking water in the UK is set by the Drinking Water Inspectorate [2]. However, the toxicity of arsenic is related to the particular species present. Inorganic forms are generally the most toxic, with arsenic(III) being more so than arsenic(V) [3]. Thus the usefulness of speciation studies can be recognised. The levels present in freshwater are generally very low but can become elevated due to a natural influx from an arsenic-rich soil or via the more likely route of contamination. Arsenic species have a number of commercial uses such as in wood preservatives, agricultural products and glass additives. Many of these applications employ the highly toxic arsenic(III) oxide, which in 1988 accounted for 98% of the worldwide arsenic consumption [4]. Contamination can thus arise from a number of sources such as use of arsenic-containing products and arsenic processing plants.

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Historically, gravimetric and titrimetric methods have been used for determining inorganic arsenic species. However, currently arsenic speciation studies are most frequently performed using liquid chromatography [5,6]. Electrophoretic methods can also be used for this task and in recent years numerous investigations using capillary zone electrophoresis (CZE) have been reported. Although conductivity detection [7] and laser-induced fluorescence detection [8] have been successfully employed, UV detection has seen the most widespread use [9,10]. One particular disadvantage with UV detection is the need to use additives to allow the detection of non-UV absorbing species. Chromate has been shown to be particularly effective for allowing indirect UV detection of arsenic species [11,12], however, chromate itself is highly toxic. As many of the applications for the analysis of arsenic use samples with only a very low level of contamination, in such systems the electrolytes used for the analysis are more toxic than the sample and thus this leads to the generation of more toxic waste than is necessary.

Isotachophoresis (ITP) is an analytical separation technique ideally suited to the analysis of inorganic ions and which offers a number of useful features compared to CZE. It is a more robust analytical technique, which can be used for samples with a wide range of analyte concentrations and thus is able to analyse microconstituents in a sample containing significant excesses of macroconstituents. For many samples only minimal pre-treatment is needed as conductivity is an appropriate detection method and there is no requirement to dissolve samples in electrolytes. Despite this, although separations have been reported for a wide range of metal ions [13,14] and small anions [15,16], little work on arsenic separations has been previously reported. Limited studies have been performed to analyse arsenic(V) using both aqueous [17] and non-aqueous [18] electrolytes, however the simultaneous determination of arsenic(III) and arsenic(V) has not been reported to the best of our knowledge.

The use of miniaturised analytical separations offers many potential benefits over the use of conventional instrumentation. Such benefits include improved analytical performance, reduced analysis times and low manufacturing costs, particularly if polymer materials are used in their construction. The advantages are now being realised and miniaturised ITP has been performed with a wide range of samples. Poly(methyl methacrylate) (PMMA) devices have been used to perform bidirectional ITP on small ions [19], analyse model mixtures of organic anions [20,21], anions in water [22], organic acids and anions in wine [23] and anions in food additives [24]. Poly(dimethylsiloxane) (PDMS) devices have been used for the separation of model mixtures of metal cations [25,26]. A further benefit of miniaturisation is the reduction in reagent and sample consumption and subsequent reduction in the quantity of waste produced, which is particularly desirable when handling toxic species such as arsenic. To date this feature has not been fully exploited although the separation of selenoamino acids using a PMMA device has been reported [27].

This paper presents an isotachophoretic method which has allowed the simultaneous determination of inorganic arsenic species. This method has been used to perform miniaturised separations of mixtures of arsenic(III) and arsenic(V) ions and also on model samples containing a range of inorganic anions. A comparison is made with the performance of the method using a capillary scale instrument. The applicability of the method to the analysis of an industrial waste water sample has also been shown using the capillary scale instrument.

2. Experimental

2.1. Microfabrication

Miniaturised ITP separation devices were produced by a technique which involved the direct milling of a 78 mm long, 78 mm wide, 6 mm thick PMMA block. Full details of the fabrication procedure have been previously reported [19]. The device produced incorporates two straight separation channels intersecting at a bifurcation point and contains a cross geometry which can be used for sample injection purposes. A schematic of the layout of the device produced is shown in Fig. 1. The flow channel from well A to the bifurcation point is 200 μm wide whereas all other channels are 300 μm wide. The depth of all channels is 300 μm. The distance from the injection cross to the bifurcation

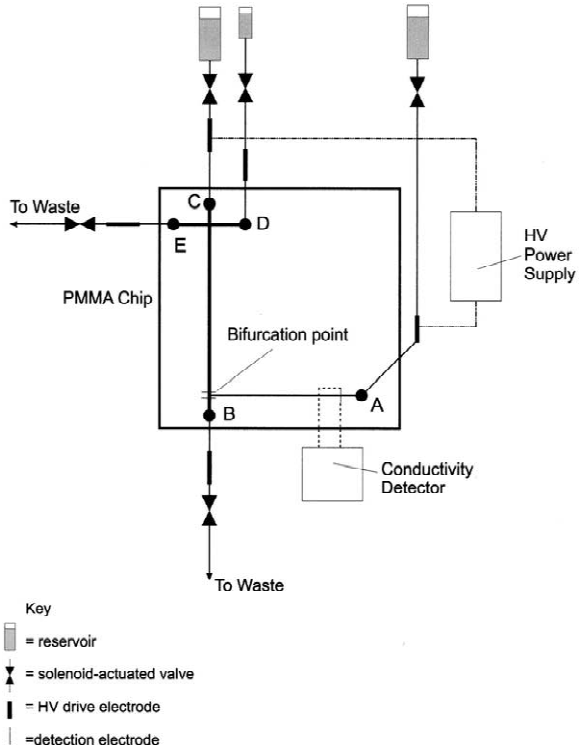

Fig. 1. Schematic diagram of the miniaturised separation device.

point is 57 mm and from the bifurcation point to detector A is 44 mm. This is the flow path used for all of the miniaturised separations performed in this work. Two additional conductivity detectors are located 2 mm away from the bifurcation point in the directions of wells B and C, respectively. All of the detectors consist of pairs of opposed on-column electrodes. The electrodes were formed of 75 μm diameter platinum wire (Aldrich, Gillingham, UK).

2.2. Instrumentation

Sample injection and movement of solutions around the separation device were achieved using a hydrodynamic sample transport system as previously reported by the authors [19]. However, in this work a change was made to the external solution reservoirs. The reservoirs for the leading and terminating electrolytes were provided by the barrels of 20-ml disposable syringes, however the sample reservoir was formed by the barrel of a 2-ml syringe allowing a reduction in the volume of sample required.

The constant currents used to drive the separations were provided by a PS350 5000 V–25 W high voltage power supply (Stanford Research Systems, Sunnyvale, CA, USA), configured to produce negative voltages. Detection was achieved using a single channel conductivity detector built in-house. The design of the detector uses capacitive coupling to isolate the detection circuitry from the high voltage used to drive the separations [28].

Control of the sample transport system, high voltage power supply and conductivity detector was achieved using LabVIEW software (version 6.1) (National Instruments, Austin, TX, USA), running under the Windows 98 operating system (Microsoft, Redmond, WA, USA) on a standard personal computer. The hardware interface is achieved using three National Instruments cards controlled using the NIDAQ driver (National Instruments) and programmed using LabVIEW code. The cards used were a PCI-GPIB board for the power supply, an AT-MIO-16E-10 multiple analogue output and input board for the detector and a Lab-PC+ multifunction card for the sample transport system.

Capillary scale separations were performed using an ItaChrom EA101 instrument (J&M Analytische Messung und Regeltechnik, Aalen, Germany). All separations were performed using a 160 mm long, 0.8 mm internal diameter fluorinated ethylene–propylene co-polymer (FEP) capillary fitted with an on-column conductivity detector. Sample injection was performed via the internal 30-μl sample loop unless stated otherwise. Control of the system and data collection were performed on a PC using ITPWin Version 2.18 software (KasComp, Bratislava, Slovak Republic).

2.3. Separation conditions

The miniaturised separations performed in this investigation were achieved using the two control programs shown in Table 1. The first step flushes the device and fills the separation channels with leading electrolyte. The timing of this step is set to ensure that all traces of previously analysed samples are fully removed from the system before performing subsequent separations. Step 2 positions the ter-

Table 1
Separation programs used for performing miniaturised ITP

Step	Program 1							Program 2						
	Time (s)	Current (μA)	Valve status					Time (s)	Current (μA)	Valve status				
			A	B	C	D	E			A	B	C	D	E
1	40	–	○	●	●	●	○	40	–	○	●	●	●	○
2	1	–	●	●	○	●	○	1	–	●	●	○	●	○
3	0.5	–	●	●	●	○	○	0.5	–	●	●	●	○	○
4	0.2	–	●	○	●	○	●	0.2	–	●	○	●	○	●
5	100	30	●	●	●	●	●	100	30	●	●	●	●	●
6	1000	20	●	●	●	●	●	100	20	●	●	●	●	●
7	–	–						1000	10	●	●	●	●	●

●, Closed; ○, open.

minating electrolyte so that the sample will be sandwiched between the leading and terminating electrolytes. Steps 3 and 4 control the amount of sample injected. The ITP separations are performed with an initial constant current of 30 μA applied between wells A and C, step 5. However, due to the low conductivity of the electrolyte systems it was found necessary to subsequently reduce this current to 20 μA to prevent bubble formation, step 6. The separation current can be further reduced to 10 μA, to allow the detection of dilute samples, step 7. Program 1 results in shorter analysis times and was used for method development and qualitative work whereas program 2 allows more dilute samples to be analysed and was used for quantitative work.

The capillary scale separations were performed using a three-step separation program. An initial separation current of 350 μA was applied for 250 s, unless otherwise stated, which was subsequently reduced to 250 μA for 70 s to prevent overheating of electrolytes. Detection was undertaken in a third step at a current of 100 μA.

2.4. Chemicals

The composition of the electrolyte system developed in this work for arsenic analysis is shown in Table 2. The chloride leading ions were provided from 1.0 *M* hydrochloric acid (volumetric standard, Aldrich, Gillingham, UK), suitably diluted. The pH buffer Tris (99.9%) and electroosmotic flow supressant hydroxyethyl cellulose (HEC) (molecular mass ca. 250 000) were supplied by Aldrich. Glycine (99%) was obtained from Sigma (Gillingham, UK), α-cyclodextrin (98%) from Fluka (Gillingham, UK) and barium hydroxide (0.05 *M* volumetric standard) from Riedel-de Haën (Gillingham, UK). Samples were produced using the following chemicals: 1000 mg l^{-1} arsenic(III) atomic absorption spectroscopy (AAS) standard solution (arsenic(III) oxide in 2%

Table 2
Composition of the electrolyte system used for performing isotachophoretic arsenic separations

	Leading electrolyte	Terminating electrolyte
Ion	Cl^-	Glycine
Concentration (mmol l^{-1})	8	10
Complexing agent	α-CD	–
Concentration (mmol l^{-1})	10	
Counter ion	Tris	Ba^{2+} [added as $Ba(OH)_2$]
pH	9.0	9.5
Additive	HEC	–
Concentration (mg ml^{-1})	1	

α-CD, α-cyclodextrin; HEC, hydroxyethyl cellulose.

(w/w) potassium hydroxide) (Aldrich), 1000 mg l^{-1} arsenic(V) standard solution (arsenic(V) oxide in 0.5 *M* nitric acid) (BDH, Poole, UK), 1000 mg l^{-1} antimony AAS standard solution (antimony potassium tartrate in water) (BDH), 100 mg l^{-1} sulphate volumetric standard solution (Aldrich), molybdenum(VI) oxide (99.5%, Aldrich), potassium tellurite hydrate (Aldrich) and potassium tellurate hydrate (Aldrich). All solutions were prepared using >18 MΩ water (Elga Maxima Ultra Pure, Vivendi Water Systems, High Wycombe, UK). The molybdenum sample stock solution was prepared by dissolving the oxide in 0.1 *M* sodium hydroxide, produced by diluting a 1.0 *M* volumetric standard solution (Aldrich).

3. Results and discussion

3.1. Electrolyte system

The primary factor that governed the choice of electrolyte system was the degree of dissociation of arsenious acid. Although arsenic(V) ions are mobile over a wide range of pH, from pH ~1 upwards, arsenic(III) ions have negligible mobility below pH 8 due to a pK_{a1} value of 9.23 [29]. Thus it was necessary to develop an electrolyte system which was buffered to a high pH. A pH of 9 was chosen because at higher pH there was thought to be a greater chance of co-migration of the arsenic(V) with other species as it fully dissociates. At high pH values the species commonly utilised as terminating electrolytes are β-alanine [18] and ε-amino caproic acid [30]. However at pH 9, these ions have very low conductivities, due to their high pK_a values of 10.24 [31] and 10.80 [29], respectively, which would restrict the size of the separation currents that could be employed and thus slow down the analyses. Thus glycine, which has a higher conductivity (pK_a 9.78 [29]), whilst still possessing a lower mobility than arsenic(III), was used as the terminating electrolyte. However, using this ion it was still found necessary to raise the conductivity of the terminating electrolyte to allow for rapid separations. Barium hydroxide was used for this purpose as it would also fulfil the role of suppressing contributions from the terminating electrolyte to the ubiquitous carbonate contamination arising from dissolved atmospheric carbon dioxide which is encountered when performing ITP at high pH [32]. To achieve the separation of arsenic(III) and arsenic(V) the addition of α-cyclodextrin is not necessary, however it is useful in assisting the separation of other anions, such as nitrate and sulphate [33].

3.2. Sample injection

Although the separation device was designed to perform injections using the cross geometry, it was found that the injection volume was too small (27 nl) to allow analysis of very dilute samples. However, the feature could be useful for the analysis of samples with higher concentration of ions, such as those that may be encountered when monitoring process streams in an industrial environment. Thus to enable the analysis of dilute samples it was necessary to use the channel from the injection cross to the bifurcation point for injection purposes. The device can then be used to inject variable amounts of sample by inserting an extra step into the separation program after the fourth step shown in Table 1. This step would involve opening valves A and E and lead to the displacement of a volume of sample, determined by the length of the step, with leading electrolyte. However, in this work the whole of this channel was used in effect as a sample loop with a volume of 5.1 μl. This enabled very reliable and reproducible sample injection to take place. For example, the RSDs in zone lengths achieved whilst performing arsenic(III) calibrations ranged from 0.9 to 8.6%. These values are better than those achieved using the capillary scale instrument in which RSD ranged from 2.4 to 13.6%. The amount of leading electrolyte supplied to the system was also found to be consistent, with the time recorded at the end of the leading electrolyte zone averaging 263±6.8 s. The error represents the standard deviation obtained from performing 12 runs using separation program 1.

3.3. Separations

The electrolyte system has been successfully used to analyse binary mixtures of arsenic(III) and arsenic(V) using miniaturised separation devices. An example of a separation containing 20 mg l^{-1} of

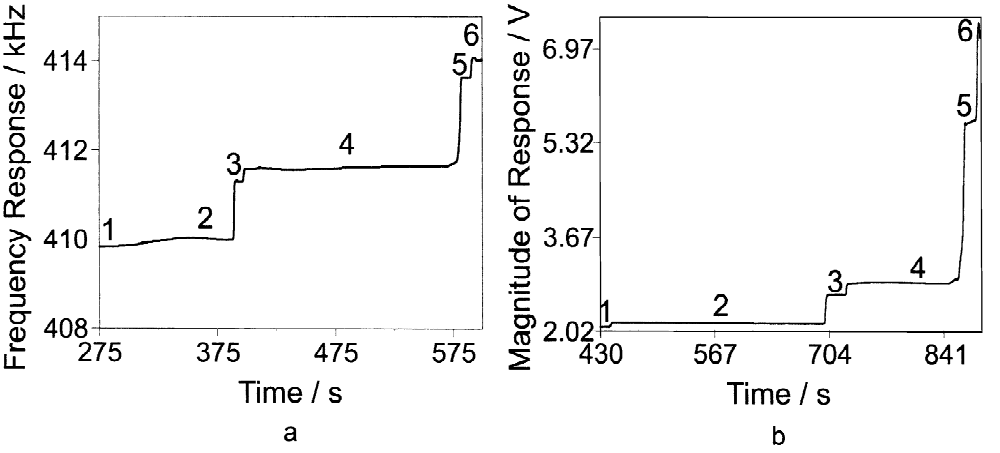

Fig. 2. Isotachopherograms of 20 mg l^{-1} samples of arsenic(III) and arsenic(V). Leading electrolyte, 8 mmol l^{-1} HCl, pH 9.0 (Tris), with 10 mmol l^{-1} α-cyclodextrin and 1 mg ml^{-1} HEC added. Terminating electrolyte, 10 mmol l^{-1} glycine at pH 9.5 [$Ba(OH)_2$]. 1, Cl^-; 2, NO_3^-; 3, AsO_4^{3-}; 4, CO_3^{2-}; 5, AsO_3^{3-}; 6, glycine. (a) A miniaturised separation achieved using separation program 1. (b) A separation performed on a capillary scale instrument.

both arsenic species, performed using separation program 1, is shown in Fig. 2a. The miniaturised device can be seen to produce a clear separation of the two species. This was expected as previous CZE separations have shown the migration times to be significantly different due to the large differences in effective mobilities in this pH region [11]. The large step between the carbonate and arsenic(III) steps suggests it is likely that the method could be extended to organic arsenic species such as dimethylarsenate and monomethylarsenate, which have electrophoretic mobilities intermediate between those of the inorganic species [9].

It can also be clearly seen in Fig. 2a that the sample is not a simple binary mixture and that two extra zones are present. One zone is carbonate contamination caused by dissolved atmospheric carbon dioxide, a common problem when performing ITP at pH values of greater than 7. The other zone present is due to nitrate, the origin of which is the arsenic(V) standard solution, which contains 0.5 mol l^{-1} nitric acid. The presence of these two unwanted zones does however illustrate how ITP can be used to analyse species in the presence of large excesses of contaminants. The zones of these species are significantly longer than those of the sample species, thus indicating significantly higher concentrations than those of the ions of interest. The nitric acid problem could be reduced quite easily by utilising nitric acid as the leading electrolyte, in which case it would not appear as a zone in its own right but would increase the length of the leading electrolyte zone. A second alternative would be to use a two-dimensional separation to send the nitrate to waste after the first dimension. The carbonate problem is much more difficult to remove and is likely to be a constant problem for the simultaneous determination of arsenic(III) and arsenic(V). A possible solution could be to find a species which would retard the arsenic(V) ions, so that they were slower than the carbonate, whilst not interacting with arsenic(III) ions. However, if there was a particular application requiring the analysis of only one of the species the problem could be simply overcome by using, for example, glycolic acid, which is slower than carbonate, as the leading electrolyte for the determination of arsenic(III) or carbonate itself as the terminating electrolyte for the determination of arsenic(V).

This ability to separate the arsenic species from large excesses of nitrate and carbonate is particularly useful as these species are ubiquitous in environmental water samples. The other major ion likely to be present in water samples in large quantities is sulphate. The presence of α-cyclodextrin in the leading electrolyte meant that the separation of the chloride leading ion, nitrate and sulphate from one

Table 3
Relative step heights of the anions analysed

Ion	Miniaturised separations			Capillary scale separations		
	RSH	RSD	*n*	RSH	RSD	*n*
SO_4^{2-}	0.042	8.1	3	0.012	8.3	3
NO_3^-	0.053	12.5	5	0.026	2.3	3
MoO_4^{2-}	0.147	2.1	3			
$Sb_2C_8H_4O_{12}^{2-}$	0.312	1.6	3			
AsO_4^{3-}	0.357	2.8	5	0.112	3.5	6
CO_3^{2-}	0.429	0.4	3	0.165	1.5	3
TeO_3^{2-}	0.622	0.9	3	0.224	1.2	3
AsO_3^{3-}	0.902	0.5	5	0.727	1.3	6

RSH, relative step height; *n*, number of runs.

another was possible. Analysis of sulphate yielded a relative step height (RSH) of 0.042, as shown in Table 3. In this work RSH was calculated using the following expression:

$$\mathrm{RSH} = \frac{h_S - h_{LE}}{h_{TE} - h_{LE}}$$

where h_{LE} is the magnitude of the response produced by the leading electrolyte (Hz [miniaturised] or V [capillary scale]); h_S is the magnitude of the response produced by the sample (Hz or V); h_{TE} is the magnitude of the response produced by the terminating electrolyte (Hz or V).

This RSH is even lower than that of nitrate, which was calculated to be 0.053, thus the presence of sulphate will not cause problems when analysing arsenic species.

For comparison purposes, Fig. 2b shows the isotachopherogram produced by the analysis of a similar sample containing 20 mg l^{-1} concentrations of both arsenic(III) and arsenic(V) achieved using the commercial capillary scale instrument. The most obvious difference between the two traces is the time taken, with the miniaturised separation taking 575 s whereas the capillary scale separation took 900 s. Indeed this analysis time for the miniaturised separation compares well with those previously reported, which have been in the range of 300 to 900 s [22,23]. It should be noted that this separation time also includes ~42 s of injection time. Much faster analysis times would also be possible were it not for the presence of the carbonate contamination zone which is over 150 s long. The zone shapes for the two arsenic species are also much sharper with the miniaturised device. Conversely the nitrate zone has a poorly defined shape possibly due to a zone of non-linearity of the in-house detector in the conductivity range of this species.

Investigations were also made into the usefulness of the method for the simultaneous analysis of other ions. Thus antimony(III), molybdenum(VI) and tellurium(IV) were investigated. These are ions which may require monitoring, as the World Health Organisation has defined the first two of these elements within their list of chemicals of health significance in drinking-water [34], and concerns have also been raised over tellurium species [12]. The species of antimony and tellurium used are the more toxic of the two common forms of these elements [35]. Separations of these ions from the inorganic arsenic species was successfully achieved. An example of a separation of a sample containing 20 mg l^{-1} arsenic(V) and arsenic(III), 25 mg l^{-1} molybdenum(VI), 30 mg l^{-1} antimony(III) and 40 mg l^{-1} tellurium(IV) performed using separation program 1 is

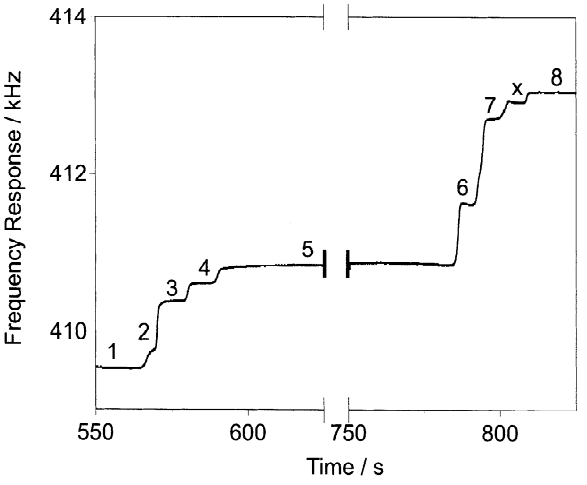

Fig. 3. Miniaturised separation of a sample containing 20 mg l^{-1} arsenic(V) and arsenic(III), 25 mg l^{-1} molybdenum(VI), 30 mg l^{-1} antimony(III) and 40 mg l^{-1} tellurium(IV) performed using separation program 1. Leading electrolyte, 8 mmol l^{-1} HCl, pH 9.0 (Tris), with 10 mmol l^{-1} α-cyclodextrin and 1 mg ml^{-1} HEC added. Terminating electrolyte, 10 mmol l^{-1} glycine at pH 9.5 [$Ba(OH)_2$]. 1, NO_3^-; 2, MoO_4^{2-}; 3, $Sb_2C_8H_4O_{12}^{2-}$; 4, AsO_4^{3-}; 5, CO_3^{2-}; 6, TeO_3^{2-}; 7, AsO_3^{3-}; 8, glycine; *x*, unknown contamination. Note the first zone shown is not that of the leading ion.

shown in Fig. 3. Note that in this figure to allow the samples of interest to be clearly seen, the major part of the nitrate contamination and consequently the end of the leading ion zone are not shown. This separation shows that the miniaturised separation device has the column capacity to allow the separation of five microconstituents and two major contaminants successfully. The analysis of tellurium(VI) should also be possible using this system. It was possible to obtain a step height for this species, in the region that was expected from the previous investigation of tellurium species using ITP by Yoshida and Hida [36], but the behaviour of the sample was found to be unstable.

A comparison in the performance of the miniaturised devices compared to the capillary scale instrument in the qualitative information obtained from the ITP separations is shown in Table 3. This table shows the RSHs for the anions studied in this investigation. It can be seen that for the majority of the species, the reproducibility in RSH values are similar or better on the miniaturised device compared to the capillary instrument. The one species that exhibits a worse performance is nitrate, with an RSD of 12.5% compared to 2.3%. The reason for this is likely to be due to the high concentrations analysed producing long, low zones which tend not to have very sharp zone boundaries with the leading electrolyte zone, and hence can be difficult to analyse.

Calibration curves were produced for the two arsenic species using both miniaturised devices, using separation program 2, and the capillary scale instrument. The details of the equations of these obtained using weighted linear regression are shown in Table 4. Good linearity was observed using the miniaturised device for both arsenic(III) and arsenic(V), with correlation coefficients of 0.996 and 0.964, respectively, achieved. Limits of detection, taken as the intercept of the calibration equation plus three times the standard deviation of this value, were calculated to be 4.8 mg l^{-1} for arsenic(III) and 1.8 mg l^{-1} for arsenic(V). These values are higher than those achieved using the capillary scale instrument which were 0.9 mg l^{-1} and 0.4 mg l^{-1}, respectively. However, if a comparison of the actual amounts analysed, rather than concentrations which can be detected is made, the miniaturised device achieves a similar performance, allowing the detection of 330 pmol of arsenic(III) and 130 pmol of arsenic(V) compared to 340 pmol and 160 pmol, respectively, with the capillary scale instrument.

One of the features of ITP is that it can analyse large volumes of sample thus allowing the analysis of very dilute samples. For example, the capillary scale instrument can be used to analyse samples with concentrations of less than 100 μg l^{-1} using this electrolyte system if manual injection of a 500-μl sample is used. However, with the current miniaturised device the results here were achieved using the maximum volume that can be injected, so design enhancements are required to enable the analysis of environmental samples which contain low concentrations of arsenic species. Such improvements should be possible as the potential to analyse sub-pmol amounts of species such as nitrite and fluoride using miniaturised ITP has been shown [22]. The design of the miniaturised devices used in this work could be improved in a number of ways to allow such separations. A larger sample loop is the most obvious modification. This change may need to be accompanied by an increase in separation channel length to prevent overloading. However, this may not be necessary as the capillary scale instrument can cope with sample volumes that are greater than six times the volume of the separation channel. The use of a narrower separation channel would also improve

Table 4
Weighted linear regression equations, of the form $y = bx + a$, for the arsenic calibration curves

		$a \pm$SD (s)	$b \pm$SD (s l mg^{-1})	r	n	Concentration range (mg l^{-1})
Miniaturised	As(III)	4.63±1.74	1.11±0.17	0.996	5	2.5–50
	As(V)	3.38±1.15	1.97±0.26	0.964	5	0.5–10
Capillary scale	As(III)	1.24±0.18	0.62±0.03	0.997	5	2.5–50
	As(V)	2.15±0.26	1.98±0.10	0.998	5	0.5–10

a, Intercept; *b*, slope; *n*, number of data points (three replications performed at each); *r*, correlation coefficient; *x*, concentration (mg l^{-1}).

limits of detection. An alternative to narrowing the entire separation channel is to taper the channel at the detector which would allow faster separations than those possible if the whole length of the channel was reduced, as higher separation currents could be used in this case.

The method has also been used to analyse an industrial process stream, known to contain arsenic, obtained from a European metal processing plant. Fig. 4 shows an example of a separation of this sample achieved using the capillary scale instrument. This result was obtained by injecting the sample, which was in the form of a colourless liquid, directly without pretreatment. A step for arsenic(V) can be clearly seen, which was the expected form of the arsenic, whereas no step is present for arsenic(III). As can be seen, the sample contains few components, as it was taken after a clean-up stage within the industrial plant. The only other step present is due to sulphate. This latter step has a poor shape due to the high concentration of sulphate present. This was expected as the sample was known to contain sulphuric acid and a number of metal sulphates. The concentration of arsenic(V) in the sample was calculated to be 0.90 ± 0.20 mg l^{-1}. The error shown represents the standard deviation based on three replicate runs. As this value is under the limit of quantification, of 1.30 mg l^{-1}, when taken as the value of the intercept plus 10 times the standard deviation of this value, the same sample was re-analysed using a manual injection with a micro-syringe in 50-μl sample volumes. The results obtained were in good agreement with those above, yielding a concentration of 0.90 ± 0.10 mg l^{-1}. This value is within the range expected as the stream can contain up to 4 mg l^{-1} of arsenic(V). This value is much higher than the Helsinki Commission—Baltic Marine Environment Protection Commission (HELCOM) limits for industrial discharges, which is 0.3 mg l^{-1} [37] and thus this stream could not be released into the environment without further clean-up or dilution.

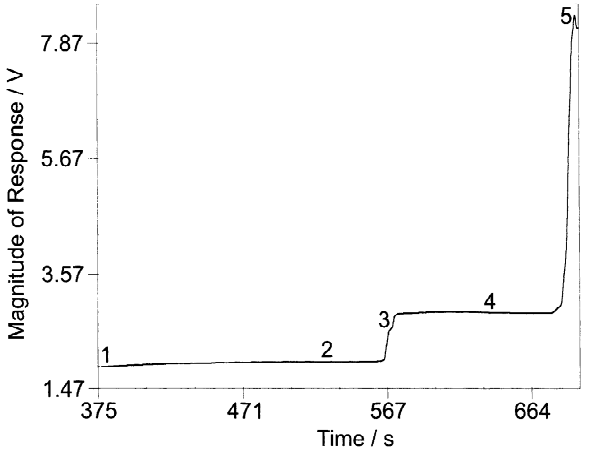

Fig. 4. Separation of an industrial waste stream using a capillary scale instrument. Leading electrolyte, 8 mmol l^{-1} HCl, pH 9.0 (Tris), with 10 mmol l^{-1} α-cyclodextrin and 1 mg ml^{-1} HEC added. Terminating electrolyte, 10 mmol l^{-1} glycine at pH 9.5 [$Ba(OH)_2$]. 1, Cl^-; 2, SO_4^{2-}; 3, AsO_4^{3-}; 4, CO_3^{2-}; 5, glycine.

4. Conclusions

These results show that isotachophoresis is a suitable technique for performing speciation studies of inorganic arsenic. The method developed for this purpose has been found to be suitable for use with miniaturised PMMA separation devices fitted with integrated conductivity detectors. The level of analytical performance of the miniaturised devices has been found to be similar to that achieved using a capillary scale commercial instrument. However, to enable the miniaturised devices to be used for applications such as the environment, design refinements are needed to allow the determination of very dilute samples.

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