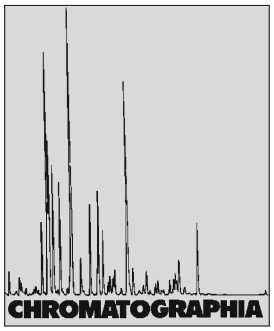
Field Enhanced Sample Injection for the CE Determination of Arsenic Compounds Using Successive Multiple Ionic Polymer Layer Coated Capillaries



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Abstract

A capillary electrophoresis method using indirect UV detection has been applied to the determination of arsenate [As(V)], arsenite [As(III)], monomethylarsonic acid and dimethylarsinic acid. The arsenic species were successfully separated in a successive multiple ionic polymer layer coated capillary. On-line sample preconcentration of arsenic compounds were performed by employing field enhanced sample injection. A baseline separation was achieved in a basic background solution of 10 mM 2,6-pyridinedicarboxylic acid at pH 10.3. The precision of migration time was 1.2–2.4% RSD and peak height was 8.1–12.9% RSD. The limits of detection at a S/N ratio of 3 for the four arsenic compounds were found to be 20–70 ppb, which are comparable to other on-line preconcentration techniques. The enhancement factor was improved by 230–1,500-fold.

Keywords

Capillary electrophoresis
Indirect UV detection
Field enhanced stacking injection
Successive multiple ionic polymer layers
Arsenic compounds

Introduction

Speciation of arsenic compounds is important due to their different toxicity levels, bioavailability, and potential for migration in the environment. The inorganic arsenic compounds, i.e., arsenate [As(V)], and arsenite [As(III)], are more toxic than the organic arsenic compounds [1]. Arsenate, arsenite, monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) are the major arsenic species found in marine water and freshwater systems. The two inorganic species, As(V) and As(III) are known contaminants of water bodies, and these species are then methylated in the environment to MMA and DMA. Arsenic species must be differentiated from one another and from the sample matrix. Sub-μg L^{-1} detection limits are required to quantify individual arsenic species. The provisional guideline value recommended by the United States Environmental Protection Agency is 0.010 mg L^{-1} in drinking water [2] which is the same as the World Health Organization guideline for arsenic in drinking water [3]. A sensitive analytical method is required for the identification and quantification of these arsenic species. The proven techniques for arsenic speciation are high performance liquid chromatography (LC) [4, 5], capillary electrophoresis (CE) [6, 7] in combination with element sensitive detectors such as atomic absorption spectrometry [8], atomic fluorescence spectroscopy [9] and inductively coupled plasma-MS [10 after hydride generation]. CE with conventional UV detection [6] suffers from poor detection sensitivity due to the

short optical path length and the small injection volume. CE with a stacking technique on a capillary coated with poly(diallydimethylammonium chloride) is reported to result in an improved sensitivity of arsenical compounds [11] but poor reproducibility in terms of migration times.

Inorganic arsenic compounds at parts per billion concentration are not detectable by UV since they have weak UV-absorbing properties. Indirect UV detection, which can be used to detect compounds that have no optical absorbance, therefore offers an alternative method for the detection of these ions. CE with indirect UV detection has been applied for the analysis of inorganic ions [12, 13] and organic acids [14]. In indirect UV detection, a UV probe is chosen as one of the components of the background electrolyte solution (BGS) creating a large background signal. When a non-absorbing ion passes through the capillary, it displaces the probe ion causing a change in the light absorption thus producing a peak [15, 16]. The highest sensitivity can be achieved if the sample ions have mobilities close to the mobility of the UV-absorbing probe. Among the probe ions, 2,6-pyridinedicarboxylic acid (PDC) and chromate have been successfully used for the determination of inorganic and organic ions [17, 18]. Chromate is not a favorable ion probe for the determination of arsenic compounds because of its limited pH range. At pH lower than 8, a precipitate forms between hydrogenchromate and the EOF modifier [18].

CE–indirect UV for the speciation of arsenic has high detection limits. Wu and Ho [19] used PDC and *n*-hexadecyltrimethylammonium hydroxide as the flow modifier to reduce the EOF. The electropherograms gave a noisy background in reference to the positive signal of the analyte peaks, and the detection limits of 0.19–0.23 ppm are still too high for its application to environmental water samples. Kitagawa et al. [20] have reported that the use of a basic BGS containing a cationic surfactant provided good separation of As(V), MMA, As(III) and DMA with CE–indirect UV detection. The LOD values were 7.8–12.5 ppm for the four arsenic species.

In order to detect anions with a lower mobility, the EOF has to be suppressed or even reversed. This can be done by coating the inner surface of the capillary with an electroosmotically non-active layer, or by a dynamic coating, i.e., by adding surfactants, such as cetyltrimethylammonium bromide (CTAB), to the BGS. Small amounts of polymeric surfactants can be added to the BGS for dynamic coating on the capillary surface [21]. A stable capillary coating using successive multiple ionic-polymer layers (SMIL) was reported by Katayama et al. [22, 23]. The SMIL coating consisted of a cationic polymer which was fixed to the capillary wall, followed by an anionic polymer and finally a third layer of cationic polymer. The third layer generates a positive charge on the capillary surface and changes the direction of the EOF. The SMIL capillary showed good stability and reproducibility.

The aim of this study was to develop an alternative speciation analysis of As(V), As(III), MMA and DMA by employing the on-line sample preconcentration technique of field enhanced stacking with indirect UV detection on an SMIL coated capillary using PDC as the background solution and as the UV probe ion in the hope that this would offer comparable detection limits to other CE methods for the speciation of arsenic compounds.

Experimental

Chemicals

Polybrene and sodium hydroxide were obtained from Wako (Osaka, Japan). PDC was obtained from Aldrich, St. Louis, Missouri, USA and dextran sulfate ($M_r = 500{,}000$) was obtained from Sigma, St. Louis, Missouri, USA. Thiourea was obtained from Fluka (St. Louis, Missouri, USA).

The arsenic standards were prepared as a stock solution of 1,000 mg L^{-1} As each from the following compounds: arsenic trioxide, As_2O_3, (M & B, Denmark), sodium arsenate, $Na_2HAsO_4{\cdot}7H_2O$, (Fluka), MMA (Chem Service, Pennsylvania, USA) and DMA (Aldrich). All reagents were of analytical grade and used without further purification. All solutions were passed through 0.45 μm filters (Nacalai Tesque, Kyoto, Japan) and sonicated prior to use.

Instrumentation

All electrophoresis experiments were performed on an HP^{3D} CE (Agilent Technologies, Waldbron, Germany) with a diode array detector and ChemStation software. Separations were performed using a homemade SMIL-coated fused-silica capillary (Polymicro Technologies, Phoenix, Arizona, USA), 50 μm I.D. × 360 μm O.D. × 72 cm effective length, with a total length of 80.5 cm. The column temperature was 25 °C. Indirect UV detection was operated at a wavelength of 350 nm with a reference wavelength of 200 nm, to enable a positive signal peak.

FESI Procedures

Samples were injected hydrodynamically (50 mbar) for 5, 10, 20 50, 100 s from the cathodic end and the separation voltage was −25 kV. Electroosmotic flow was measured with thiourea. Deionized water was purified with a Milli-Q system from Millipore (Bedford, Massachusetts, USA). Conductivities of samples and BGS were measured using a Horiba ES-12 conductivity meter (Kyoto, Japan), and the pH values were measured with a Mettler Toledo MP220 pH meter (Columbus, Ohio, USA).

For the FESI, a water plug was injected hydrodynamically at various lengths before the samples were injected electrokinetically. The length of water plug and samples injected were investigated at different pressures, voltages and times. The FESI condition used was an injection of a water plug at 50 mbar for 20 s, followed by the injection of the samples at −20 kV for 10 s.

SMIL-Coated Capillary

A modified coating procedure [22] was employed whereby a freshly cut capillary was rinsed with 1 M NaOH followed by

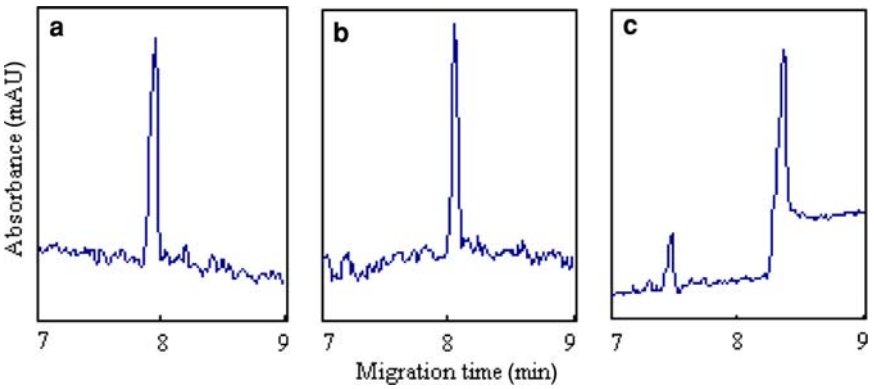

Fig 1. Effect of BGS pH on the peak shape of 10 ppm As(III). Sample was injected at 50 mbar for 2 s. Indirect UV detection was at a signal wavelength of 350 nm and a reference wavelength of 200 nm. **a** = pH 5.0 **b** = pH 10.3 **c** = pH 11.5

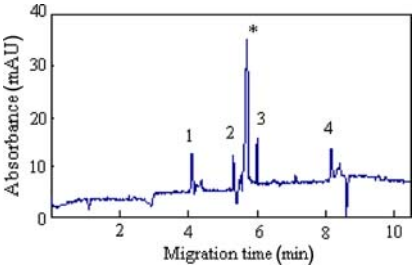

Fig 2. Field enhanced stacking separation of arsenic compounds using a BGS of 10 mM PDC adjusted to pH 10.3. Detection was at a signal wavelength of 350 nm and a reference wavelength of 200 nm. Peaks identification: **1** = As(V), **2** = MMA, **3** = DMA, **4** = As(III), * = system peak

deionized water for activation. The first layer of the capillary was coated with a 10% polybrene solution to provide a cationic coating layer. The second layer of an anionic coating used a 3% dextran sulfate solution, followed by the third layer of 10% polybrene solution. Different coating times and standing times after each coating procedures influenced the performance of the SMIL capillaries.

CE–Indirect UV Analysis

A BGS was prepared in PDC, and the pH was adjusted with 1.0 M NaOH. A solution containing the four arsenic compounds was also adjusted to pH 10 with 1.0 M NaOH. Before analysis, a new SMIL-coated capillary was conditioned by flushing with the BGS for 30 min and water (10 min). Between consecutive runs, the capillary was flushed with water (3 min) and BGS (5 min) to ensure no carry-over. All rinses were carried out at ca. 1 bar.

Results and Discussion

Effect of pH of Background Electrolyte Solution

The migration behaviors of the arsenic compounds are dependent on their pK_a values and thus the buffer pH of the BGS plays a major role in the separation of these compounds. The arsenic compounds in this study are polyprotic acids, and their apparent charge is dependent on both their pK_a and BGS pH values. Experiments were conducted with the PDC to determine if it could be used at a higher pH. The effect of pH of the background solution was initially investigated at pH range of 5–12 for As(III) because this arsenic species changes from being an undissociated acid to a dissociated state in this pH range. Increasing pH will enhance the ionization of the arsenic compounds. The alkaline pH also ensures that all the arsenic compounds are in the anionic form to promote migration to the anode. The pH effect on As(III) analysis was investigated at these pHs because As(III) may be oxidized to As(V) under high pH conditions [24] and at pH values below 9, As(III) is unprotonated [As(III) pK_a 9.3, 11.2].

Figure 1 shows the effect of BGS pH on As(III) analysis. The peak showed a little fronting when low pH of the BGS was employed but the peak became narrower as the pH was increased. The change in peak width is ascribed to the changes in the electrophoretic mobilities of both analyte ions and buffer ions. However, the peak was split into two when the pH was higher than 11 (Fig. 1c), possibly due to the oxidation of As(III) to As(V) under high pH conditions. A pH of 10.3 was observed to give a stable and reproducible peak shape for As(III) and was therefore employed in further experiments.

Field Enhanced Stacking Mode

A solution of 250 ppm each of the four arsenic compounds in pure water was injected by pressurizing for 2 s at 50 mbar. After sample injection, a −25 kV was applied for the sample stacking and separation. Figure 2 shows the electropherogram for the simultaneous separation of the four arsenic species. The sample solution has a lower conductivity than the BGS and when the voltage is applied, the analyte ions experience an increased electrophoretic mobility and migrate rapidly out of the sample zone and into the electrolyte zone and are concentrated along the boundary between the two zones. As(V) (pK_{a1} = 2.3), which is highly ionized at the pH of the BGS, migrated earliest followed by the other arsenic compounds, MMA (pK_{a1} = 3.6), DMA (pK_{a1} = 6.2), and arsenite, As(III) (pK_a = 9.3). The migration order is consistent with their pK_a values, indicating the existence of these arsenic compounds as anions at this pH. A huge system peak was observed between the MMA and DMA peaks.

The limits of detection (LOD) and relative standard deviations for CE–indirect UV detection of the arsenic species are described below. An LOD range of 1.8–10.5 ppm was obtained with As(III) showing the lowest sensitivity with a slightly broadened and tailing peak shape. Good precision was obtained with the RSD of migration time of 1.5–2.4%, and RSD of peak

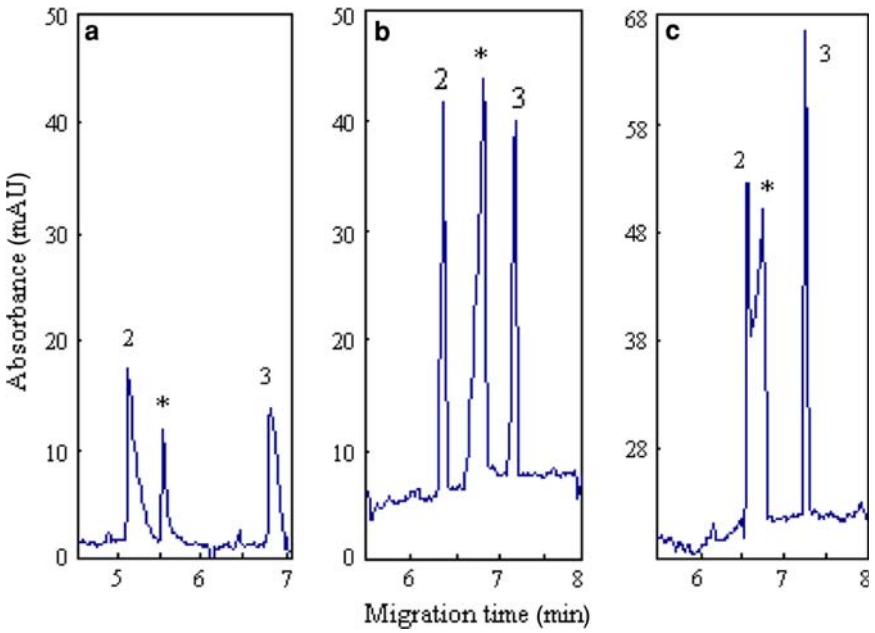

Fig 3. Effect of PDC concentration on the sensitivity. A water plug was injected at 50 mbar for 20 s and the sample injected at −20 kV for 10 s. **a** 5 mM PDC, **b** 10 mM PDC, **c** 15 mM PDC. Peak identification: **2** = MMA, **3** = DMA, * = system peak

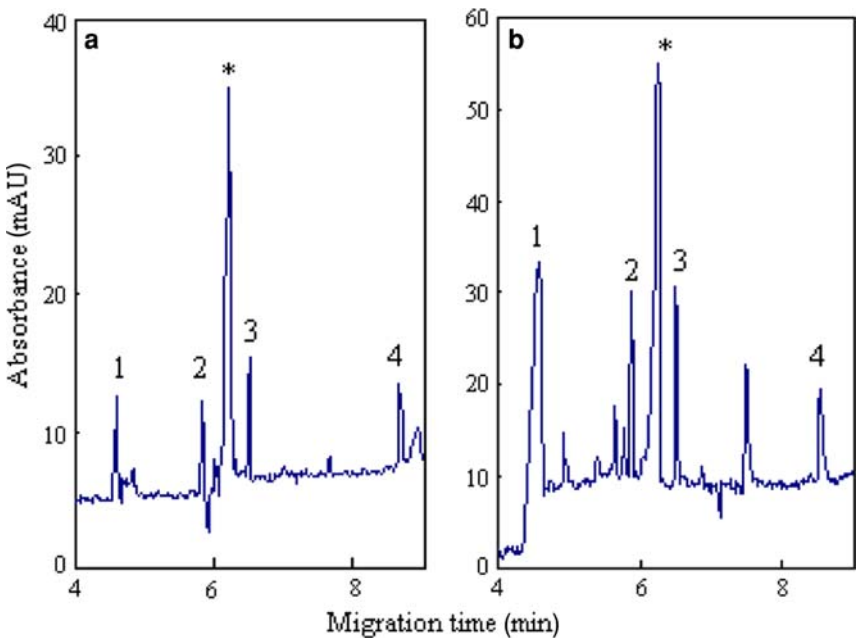

Fig 4. Comparison between conventional and FESI method. **a** 5 mM PDC, pH 10.3, sample injection: 50 mbar, 2 s. **b** 10 mM PDC, pH 10.3, water plug injection: 50 mbar, 20 s; sample injection: −20 kV for 10 s. Separation voltage −25 kV, detection wavelength: 350 nm and reference wavelength: 200 nm. Peak identification: **1** = As(V), **2** = MMA, **3** = DMA, **4** = As(III), * = system peak

height of 2.1–4.9%. These results showed that CE–indirect UV can be applied to the speciation of arsenic compounds.

Concentration of Probe Ion

Insufficient concentration of the probe ion, PDC could cause the low sensitivity of the As(III) signal. Higher sensitivity could be expected if the concentration of the probe ion were optimized. Sensitivity and peak shapes were examined with 5, 10 and 15 mM PDC solutions employing the FESI, and the results are shown in Fig. 3. Sensitivity was increased as the PDC solution concentration increased but when 15 mM PDC was employed the MMA peak and the system peak co-migrated. This is mainly caused by the change in migration velocity of the analyte but the system peak is not concentrated. The best peak shape and sensitivity was observed with 10 mM PDC.

Field Enhanced Sample Injection

Repeatability of migration times and peak areas is often a problem in CE due to the small injection volume. High concentration efficiency can be expected when the difference in conductivity between the BGS and a water plug is examined. Electrokinetic injection utilizing a water plug was investigated [25]. Sample stacking was achieved with FESI with a longer water plug and higher injection voltage. The best condition obtained was −20 kV for a 10 s injection of the samples after an injection of a water plug at 50 mbar for 20 s. The four arsenic species were analyzed at these optimum conditions and the results are shown in Fig. 4 in comparison to the conventional method.

The limit of detection (LOD), sensitivity enhancement factor (SEF_{height}) and % RSD are described below. The sensitivity enhancement factors (SEF_{height}) were calculated by dividing the peak intensities obtained with FESI by the intensities from the conventional injection of 50 mbar, 2 s, and correcting for the dilution factor. An enhancement in sensitivity of 230–1,500-fold was obtained for the four arsenic compounds. The limit of detection was between 20 and 70 ppb. These LOD values are better than previous reported studies using CE–indirect UV [19], slightly better than a capillary coated with poly(diallydimethylammonium chloride) [11], and large-volume sample stacking with polarity switching [26]. The RSD of

migration time was 1.2–2.4%, comparable to the field enhanced stacking method. However, the RSD of peak height was 8.1–12.9%, three times higher compared to the field enhanced stacking method, although the values are still within the acceptable range.

Conclusion

A CE–UV indirect analysis method has been successfully applied for the speciation of arsenic compounds by using an SMIL-coated capillary. The field enhanced sample injection on-line preconcentration technique can be applied to the arsenic compounds, producing up to a 1,500-fold increase in sensitivity with LODs of 20–70 ppb. The only problem encountered was the preparation of the SMIL coating which was not always reproducible; however, commercial SMIL capillaries are available for routine use. The BGS made of PDC solution alone does not have a good buffering capacity and caused a fluctuation in the current, and this necessitates the periodical changing of the inlet and outlet buffer solutions. The addition of tris (hydroxymethyl)aminomethane (TRIS) to control the buffer pH could provide better results with small drift of migration times expected.

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