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The Ion-chromatographic Behavior of Arsenite, Arsenate, Methylarsonic Acid and Dimethylarsinic Acid on the Hamilton PRP-X100 Anion-exchange Column

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The HPLC separation of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid has been studied in the past but not in a systematic manner. The dependence of the retention times of these arsenic compounds on the pH of the mobile phase, on the concentration and the chemical composition of buffer solutions (phosphate, acetate, potassium hydrogen phthalate) and on the presence of sodium sulfate or nickel sulfate in the mobile phase was investigated using a Hamilton PRP-X100 anion-exchange column. With a flame atomic absorption detector and arsenic concentrations of at least 10 mg dm^{-3} all investigated mobile phases will separate the four arsenic compounds at appropriate pH values in the range 4–8. The shortest analysis time (~3 min) was achieved with a 0.006 mol dm^{-3} potassium hydrogen phthalate mobile phase at pH 4, the longest (~10 min) with 0.006 mol dm^{-3} sodium sulfate at pH 5.9 at a flow rate of 1.5 cm^3 min^{-1}. With a graphite furnace atomic absorption detector at the required, much lower, flow rate of ~0.2 cm^3 min^{-1} acceptable separations were achievable only with the pH 6 phosphate buffer (0.03 mol dm^{-3}) and the nickel sulfate solution (0.005 mol dm^{-3}) as the mobile phase. To become detectable approximately 100 ng arsenic from each arsenic compound (100 µl injection) must be chromatographed with the phosphate buffer, and approximately 10 ng with the nickel sulfate solution.

Keywords: Arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, anion-exchange chromatography, arsenic-specific detectors, flame atomic absorption spectrometry, graphite furnace atomic absorption spectrometry

INTRODUCTION

Arsenic, essential for animals[1,2] and popularly acknowledged for its toxic properties, is ubiquitous. Arsenite and arsenate are present in surface waters, in groundwaters, in soils, in plant tissues and in animal tissues.[3] Biologically mediated methylation reactions convert arsenite to methylarsonic acid and dimethylarsinic acid.[4] For the study of the abiotic and biotic transformations of arsenic compounds and for the evaluation of risks associated with the exposure of biological systems to various arsenic compounds, precise and accurate methods for the identification and quantification of arsenic compounds must be available. Among the many methods for the determination of inorganic and organic arsenic compounds,[5] high-pressure liquid chromatography using anion-exchange columns, cation-exchange columns, normal-phase silica gel columns, reversed-phase columns, or gel-permeation columns provide much versatility. In addition to the large selection of stationary phases, the composition of the mobile phase can be appropriately controlled (aqueous–organic solvent mixtures, pH, buffer solutions, solutes in the mobile phase) to optimize the separation. A large number of stationary/mobile phase combinations are reported in the literature for the separation of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid.[5,6] A systematic investigation of the influence of pH and dissolved solutes in the mobile phase on the separation of arsenic compounds has not yet been carried out. This paper focuses on the dependence of the separation of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid on the Hamilton PRP-X100 anion-exchange column, on the pH of the mobile phase, on the nature of the buffer substances, and on the presence of salts dissolved in the mobile phase. Following custom-

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ary usage, the terms arsenite, arsenate, methylarsonic acid, and dimethylarsinic acid have a generic meaning and do not provide information about the degree of protonation or deprotonation of these species.

EXPERIMENTAL

Chemicals

$NaAsO_2$, $Na_2HAsO_4 \cdot 7H_2O$, $NiSO_4 \cdot 6H_2O$, potassium hydrogen phthalate (KHP), 100% acetic acid, Na_2SO_4, $Na_2HPO_4 \cdot 2H_2O$, and $NaH_2PO_4 \cdot H_2O$ (all p.a. quality) were purchased from Merck. $CH_3COONa \cdot 3H_2O$ of p.a. quality was purchased from Fluka. Methylarsonic acid (m.p. 156 °C) was recrystallized from methanol. Dimethylarsinic acid (m.p. 190 °C) was dried over phosphorus pentoxide. Water for preparing stock solutions and mobile phases was distilled three times in a quartz distillation apparatus (Destamat, Heraeus).

Solutions

Stock solutions of the arsenic compounds containing 50 mg dm^{-3} arsenic (0.66 mmol dm^{-3}) were prepared by dissolving 21.5 mg $NaAsO_2$, 52.0 mg $Na_2HAsO_4 \cdot 7H_2O$, 23.6 mg methylarsonic acid or 22.8 mg dimethylarsinic acid to 250 cm^3. With micropipettes (Transferpette, Brand, Germany) aliquots (100–300 µl of the arsenite solution, 600–3600 µl of the other solutions) were transferred to a 10 cm^3 volumetric flask and diluted to the mark to obtain a solution for chromatography with 0.05–0.15 µg arsenic (for arsenite) and 0.3–1.8 µg arsenic (for the other arsenic compounds) in the injected volume of 100 µl.

Solutions of 0.03 mol dm^{-3} NaH_2PO_4 and 0.03 mol dm^{-3} Na_2HPO_2 were mixed in appropriate ratios to obtain mobile phases with pH values from 5 to 8 (Orion SA 720 pH-meter). The 0.006 mol dm^{-3} phosphate buffer solution was prepared by diluting the 0.03 mol dm^{-3} phosphate buffer solution with distilled water. Solutions of nickel sulfate (0.006 mol dm^{-3}, 0.005 mol dm^{-3}) and sodium sulfate (0.006 mol dm^{-3}) were prepared in triply distilled water. The potassium hydrogen phthalate mobile phases (0.006 mol dm^{-3}) were prepared by dissolving potassium hydrogen phthalate in triply distilled water followed by adjustment of the pH by dropwise addition of 15% KOH. The pH was checked with a pH-meter. Acetate buffers (0.03–0.06 mol dm^{-3}) were prepared by mixing solutions of sodium acetate and acetic acid of the same molarity in the appropriate ratios to obtain buffer solutions in the pH range 4–6. The pH of these solutions was checked with a pH-meter.

Instrumentation

The HPLC system consisted of a Milton Roy CM 4000 multiple solvent delivery unit and a PRP-X100 anion-exchange column (Hamilton, Reno, Nevada, USA; 25 cm × 4.1 mm i.d.; spherical 10 µm particles of a styrene–divinylbenzene copolymer with trimethylammonium exchange sites; stable between pH 1 and 13; exchange capacity 0.19 meq g^{-1}). A 100 µl loop was used in conjunction with a Rheodyne six-port injection valve. A guard cartridge (Hamilton, Reno, Nevada, USA) filled with the same stationary phase protected the analytical column. A Hitachi Z-6100 flame atomic absorption spectrophotometer (FAA) or a Hitachi Z-9000 Zeeman graphite furnace atomic absorption spectrometer (GF AA) was used to detect the arsenic compounds in the column effluent.

HPLC–FAA

The HPLC column exit was connected to the FAAS nebulizer with a steel capillary 1 m long, 0.23 mm i.d. The FAA was operated with an acetylene/air flame at a fuel pressure of 22 kPa acetylene and 160 kPa air. The optimal height of the burner head for arsenic monitoring was 5 mm. The hollow cathode lamp (S&J Juniper, Essex, UK) was operated at 10 mA. Arsenic was measured at 193.7 nm. Data were transferred to a personal computer via a Hitachi recorder interface (part no. 171-9124) after analog-to-digital conversion. The data were treated with a modified version of a computer program published elsewhere.[7]

HPLC–GF AA

A Brinckman-type[8] flow-through cell (well volume 200 µl) was placed in the circular hole drilled into the steel base exactly under the No. 1 sample position of the sample holder (sample tray and tray cover removed) of the Z-9000. The

column exit was connected to the bottom of the flow-through cell via a steel capillary 1 m long, 0.23 mm i.d., and a Teflon tube fitted to the steel capillary with an Omnifit Teflon two-way connector. A Teflon tube at the top of the well removed effluent to a water aspirator and maintained flow-through conditions. The autosampler arm transferred every 75 s 20 µl of the effluent from the well of the flow-through cell into the graphite cuvette (from Ringsdorff Werke GmbH, Bonn, Germany; highest-purity graphite, type RWO, shape RWO 521). Argon (99.999%) was used as sheath gas (3 $dm^3 min^{-1}$) and as carrier gas (200 $cm^3 min^{-1}$; 30 $cm^3 min^{-1}$ during atomization). The arsenic hollow-cathode lamp (Cathodeon Ltd, Cambridge, UK) was operated at 8 mA. The injected aliquots of the effluent were dried at temperatures rising from 50 °C to 200 °C within 5 s, kept at 200 °C for 20 s, ashed at 300 °C for 5 s and atomized at 2600 °C for 5 s. The cuvette was cleaned at 3000 °C for 3 s.

Chromatography

Determination of the retention times of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid with various mobile phases using FAA detection

The column was equilibrated by passing 100 cm^3 of each mobile phase through the column before injection of the arsenic species. In the case of the acetate buffer, 200 cm^3 had to be passed through the column to obtain reproducible retention times. Aliquots (100 µl) of the solutions of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid containing 5 µg arsenic from each compound were chromatographed separately at 25 °C with all mobile phases at a flow rate of 1.5 $cm^3 min^{-1}$. Each retention time was determined three times (relative standard deviation <1%).

Ion-chromatographic separation of arsenite, arsenate, methylarsonic acid, and dimethylarsinic acid with various mobile phases and GA AA detection

After equilibration of the column with 100 cm^3 of the mobile phase (for the acetate mobile phase 200 cm^3), a solution (100 µl) containing all four arsenic species (50–150 ng arsenic as arsenite, 300–1800 ng arsenic each of the other compounds) was injected at 25 °C. The flow rate was 0.2 or 0.15 $cm^3 min^{-1}$.

RESULTS AND DISCUSSION

The presence of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid in many biological systems brought about the need for analytical methods that allow the identification and quantification of these arsenic compounds in complex matrices. A widely used method for this purpose is liquid chromatography with anion-exchange, cation-exchange or reversed-phase stationary phases.[5,6] Aqueous solutions of carbonates, phosphate buffers, acetate buffers or water/organic solvent mixtures with ion-pairing reagents were used as mobile phases in isocratic or gradient modes.[5] Systematic investigations to find optimal conditions for the separation of arsenic compounds have not been carried out. A mobile phase ideally should separate all arsenic compounds in a reasonable time under isocratic conditions. The mobile phase should be compatible with arsenic-specific detectors, such as FAA, GF AA, and inductively coupled plasma spectrometers. For all these detectors, aqueous mobile phases without organic components are preferable. Many arsenic compounds are acids and the negative charge on the species and thus their interaction with anion-exchange resins will be determined by the pH of the mobile phase. The anions derived from substances dissolved in the mobile phase (buffer components, neutral salts) will compete with the analyte anions for the exchange sites and thus influence the retention times and the separation of the arsenic compounds.

Arsenous acid (H_3AsO_3), arsenic acid (H_3AsO_4), methylarsonic acid ($CH_3AsO_3H_2$), and dimethylarsinic acid [$(CH_3)_2AsO_2H$] will deprotonate with increasing pH and become negatively charged. The distribution diagram (Fig. 1) informs about the species present in aqueous solution at pH values in the pH range 0–14. The 'apparent charge' [$\Sigma n \times$ (concentration of anion with charge $n-$ /analytical concentration of analyte] on these species as a function of pH is presented in Fig. 2. These diagrams show that the four arsenic compounds are fully protonated and present in solution as neutral molecules at pH 1 and completely deprotonated and thus negatively charged (arsenite 1−, dimethylarsinic acid 1−, methylarsonic acid 2−, arsenate 3−) at pH values of 12 and higher. Good separations should be achievable at pH values at which the differences among the apparent charges for the four compounds are as large as possible. This condition is

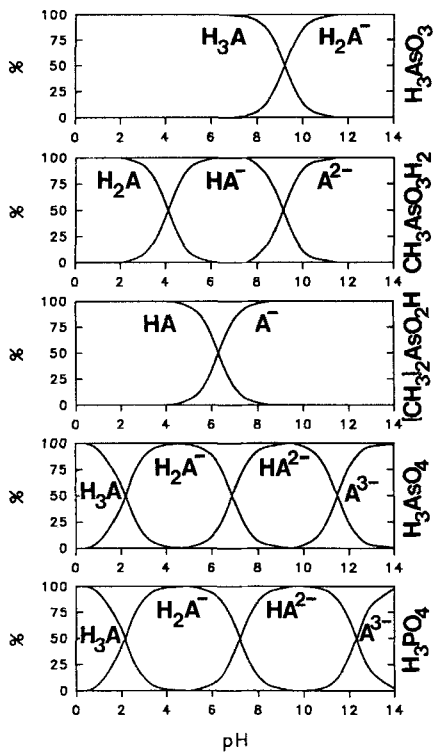

Figure 1 Species distribution diagram for arsenous acid, methylarsonic acid, dimethylarsinic acid, arsenic acid and phosphoric acid in the pH range 0–14.

met at pH values betwen 6 and 10 (Fig. 2). To maintain pH values of solutions in this range, buffers should be used. The anions of the buffer will compete with the analyte anions for exchange sites on the stationary phase and thus influence the retention times of the analytes. To elucidate the chromatographic behavior of the four arsenic compounds under these conditions, several buffer solutions (phosphate, acetate, KHP buffers) and simple salt solutions (sodium sulfate, nickel sulfate) were used as mobile phases. Most of the experimental results can be interpreted on the basis of electrostatic interactions between the anions in solution and the cationic sites on the stationary phase. With methylarsonic acid and dimethylarsinic acid hydrophobic interactions between the methyl groups and the organic backbone of the anion-exchange material must also be considered.

The following mobile phases were investigated: 0.03 mol dm^{-3} acetate buffers at pH 4–6; 0.006 mol dm^{-3} potassium hydrogen phthalate solutions at pH 4–6; 0.03 mol dm^{-3} phosphate buffers at pH 5–8; 0.006 mol dm^{-3} solutions of sodium sulfate; and 0.006 mol dm^{-3} solutions of nickel sulfate. With some of these mobile phases the concentration of the dissolved salts was also varied.

Chromatographic behavior of the arsenic compounds

Arsenite

The retention time for arsenite (~95 s) is independent of the nature of the mobile phases investigated, of the pH in the range 4–8 (Figs 3, 4), and of the concentration of the buffer solutions. This concentration independence is shown in Fig. 5 for the acetate mobile phases. Arsenite migrates with the solvent front. This behavior is expected, because arsenite is present as neutral H_3AsO_3 (pK 9.2) throughout the pH range 4–8 (Fig. 1) and does not interact with the stationary phase.

Dimethylarsinic acid

With a pK of 6.3, dimethylarsinic acid is present as a neutral molecule at pH values less than 5 (Fig. 1). Therefore, this compound is also expected to migrate with the solvent front.

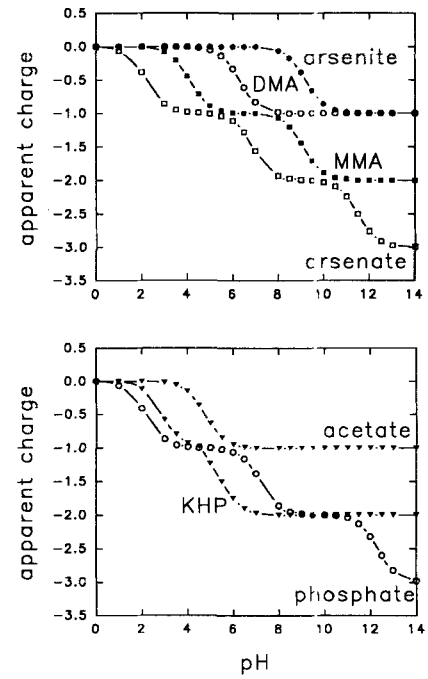

Figure 2 Apparent charges on arsenite, dimethylarsinic acid, methylarsonic acid, arsenate, acetate (pK 4.75), potassium hydrogen phthalate (pK_1 2.89, pK_2 5.51) and phosphate (pK_1 2.16, pK_2 7.2, pK_3 12.32) as a function of pH.

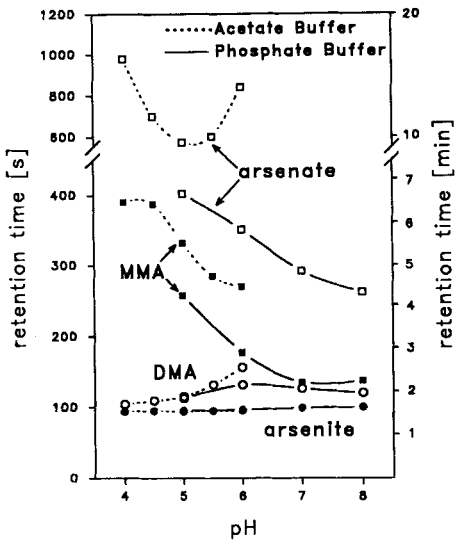

Figure 3 Dependence of the retention times of arsenate, methylarsonic acid, dimethylarsinic acid and arsenite on pH with acetate- or phosphate-buffered aqueous mobile phases (0.03 mol dm^{-3}) at a flow rate of 1.5 cm^3 min^{-1} (detector: FAA).

However, dimethylarsinic acid has retention times at these pH values that are 10–20 s longer than the retention time for uncharged arsenous acid. Because the retention cannot be caused by electrostatic interactions, the methyl groups of dimethylarsinic very probably interact hydrophobically with the organic backbone (styrene/divinylbenzene polymer) of the stationary phase. Such an interaction was also postulated for the explanation of the retention of dimethylarsinic acid on a strong cation-exchange column.[9]

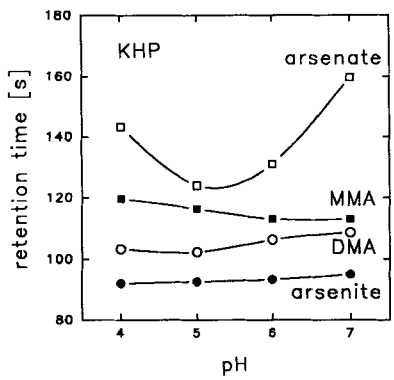

Figure 4 Dependence of the retention times of arsenate, methylarsonic acid, dimethylarsinic acid and arsenite on pH with a potassium hydrogen phthalate-buffered aqueous mobile phase (0.006 mol dm^{-3}) at a flow rate of 1.5 cm^3 min^{-1} (detector: FAA).

Above pH 5, dimethylarsinic acid begins to deprotonate. The apparent charge increases from 0.05– at pH 5 to almost 1– at pH 8 (Fig. 2). The anion will interact with the ammonium groups on the stationary phase with a concomitant increase of retention time. At pH 6 the retention times are 156 s with the acetate, 131 s with the phosphate, and 106 s with the phthalate mobile phase. The higher the apparent charge on the anion of the mobile phase (acetate, KHP, phosphate), the faster dimethylarsinate will be moved through the column. The apparent charges at pH 6 are 0.95– for acetate, 1.06– for phosphate, and 1.75– for KHP. With increasing apparent charges on the 'buffer' anions, the retention time for dimethylarsinic acid decreases (Figs 3, 4).

Between pH 6 and 8 the apparent charge on dimethylarsinic acid increases from 0.33– to 0.98–, and on phosphate from 1.06– to 1.86–. On the basis of apparent charge, the retention time for dimethylarsinic acid should increase in the pH range 6–8. However, the charge on the phosphate increases to almost 2–, allowing the phosphate to compete more effectively with dimethylarsinate for the exchange sites. The combination of these two trends leads to a decrease of the retention time for dimethylarsinic acid from 131 s at pH 6 to 120 s at pH 8 (Fig. 3).

In this pH range KHP has the highest apparent charge (1.75– at pH 6, 1.97– at pH 8) among the investigated buffer substances, allowing only a very small increase of the retention time for dimethylarsinic acid from 106 to 108 s in spite of the increase in apparent charge from 0.33– to 0.98– for dimethylarsinic acid. The great eluting

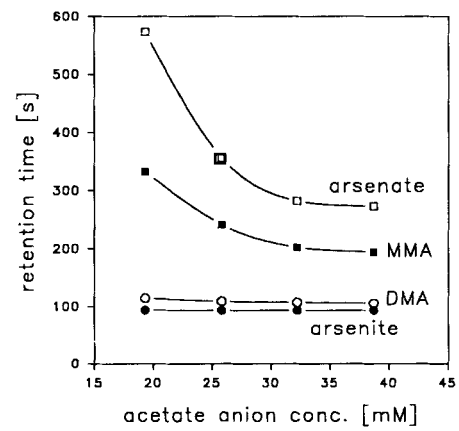

Figure 5 Dependence of the retention times of arsenate, methylarsonic acid, dimethylarsinic acid and arsenite on the acetate ion concentration of the acetate buffer (pH 5) used as mobile phase at a flow rate of 1.5 cm^3 min^{-1} (detector: FAA).

power of KHP solutions required the use of 0.006 mol dm^{-3} solutions (one-fifth the concentration of the acetate and phosphate solutions). At higher concentrations of KHP the retention times of the arsenic compounds moved towards the solvent front.

Electrostatic interactions between anions and the cationic sites on the stationary phase are much stronger than hydrophobic interactions. Therefore, hydrophobic interactions should not influence the retention time of dimethylarsinic acid above pH 5.

Methylarsonic acid

The retention times of methylarsonic acid decrease with increasing pH for all three mobile phases (Figs 3, 4). Whereas the decrease is pronounced for the acetate mobile phase (390 s at pH 4, 270 s at pH 6) and the phosphate mobile phase (257 s at pH 5, 137 s at pH 8), only a small decrease is observed for the KHP mobile phase (120 s at pH 4, 113 s at pH 7). On the basis of the pK values for methylarsonic acid (4.1, 9.1) an increase and not a decrease of the retention times with increasing pH is expected, because the apparent charge on methylarsonic acid is 0.44− at pH 4 and close to 1− in the pH range 5–8.

The observed decrease in retention times with the acetate buffers as mobile phases must be caused by the concentration of the acetate anions, which increases by a factor of six in the pH range 4 (4.5 mmol dm^{-3}) to 6 (28.5 mmol dm^{-3}). This concentration increase shifts the equilibrium (Eqn [1]) towards the right, resulting in shorter retention times.

$$CH_3AsO_3H^-_{stat} + CH_3COO^-_{soln} \rightleftharpoons CH_3AsO_3H^-_{soln} + CH_3COO^-_{stat} \quad [1]$$

That the retention times decrease with increasing acetate concentration was verified in experiments with acetate buffers of pH 5 as mobile phases, in which the acetate concentration was changed from 19.2 mmol dm^{-3} to 38.4 mmol dm^{-3} (Fig. 5).

The observed dependence of the retention time on the pH is the composite of the increase expected on the basis of the increasing degree of deprotonation with increasing pH (this effect is most important in the pH reange 4–5) and the decrease with increasing concentration of acetate that overcompensates the effect of deprotonation.

With phosphate buffers in the pH range 5–8 the retention times for methylarsonic acid decrease from pH 5 to pH 7 and remain constant between pH 7 and 8 (Fig. 3). The pK values of methylarsonic acid indicate that the monoanion is the main species present over the whole pH range from 5 to 7. The decrease of retention time in the pH range 5 to 7 can be rationalized by the increase of the ratio $HPO_4^{2-}/H_2PO_4^-$ and the increase of the HPO_4^{2-} concentration with increasing pH of the mobile phase. The doubly charged HPO_4^{2-} has a higher affinity for the positively charged groups at the surface of the stationary phase than the singly charged methylarsonate. A more detailed discussion of the changes with respect to the phosphate ions is given in the section on arsenate.

KHP solutions (0.006 mol dm^{-3}) as mobile phases produced the shortest retention times and the smallest decrease in retention times (Fig. 4) for methylarsonic acid among the three mobile phases investigated. The great eluting power of the KHP solutions can be understood in terms of the apparent charges that are always larger than the apparent charges on the methylarsonic acid over the pH range 4 to 7. Because methylarsonic acid is never present as a protonated species over the pH range investigated, hydrophobic interactions similar to interactions postulated for dimethylarsinic acid cannot be important and were not observed.

Arsenate

The retention times of arsenate show two types of pH dependencies. With the acetate (Fig. 3) and the KHP (Fig. 4) mobile phases, the retention times reach a minimum approximately at pH 5. With the phosphate buffer the retention time decreases monotonical from pH 5 to 8 (Fig. 3). The retention times are highest with the 0.03 mol dm^{-3} acetate buffers (570–980 s), intermediate for the 0.03 mol dm^{-3} phosphate buffers (260–400 s), and shortest for the 0.006 mol dm^{-3} KHP buffers (124–160 s).

With 0.03 mol dm^{-3} acetate buffers the pH of the mobile phases can be adjusted in the pH range 4–6. The apparent charge on the arsenate is almost constant (~1−) between pH 4 and 5. The apparent charge on the acetate (and thus the concentration of the acetate ion) increases in this pH range from 0.15− to 0.64− (Fig. 2). With increasing apparent charge on the acetate, the acetate ions compete more effectively with $H_2AsO_4^-$ for the positive exchange sites and, thus, gradually decrease the retention time of arsenate.

The decrease of retention times with increasing acetate concentrations at a constant pH of 5 (Fig. 5) supports this explanation. At pH 5.5 the apparent charge on the arsenate begins to increase again, reaching 1.1− at pH 6. The apparent charge on acetate cannot exceed 1− (a value reached at pH 6) and is always smaller than the apparent charge on arsenate. The higher apparent charge on the arsenate as compared with acetate explains the increase in retention time between pH 5 to 6.

The pH dependence of the retention time for arsenate with KHP solutions as mobile phases can be rationalized in the same manner as for the acetate mobile phases. The apparent charges on KHP in the pH range 4–7 are more negative by approximately one unit in comparison with acetate. This higher negative charge leads to the short retention times of arsenate (124–160 s) for the KHP mobile phases.

On the basis of the pK values for arsenic acid (2.2, 6.9, 11.5) an increase and not a decrease of retention times with increasing pH would be expected for arsenate. The phosphate buffer in the mobile phase introduces dihydrogen phosphate and monohydrogen phosphate anions. Both phosphate anions will compete with the analyte anions for the positively charged groups at the surface of the stationary phase. The ratio $HPO_4^{2-}/H_2PO_4^-$ (determining the pH and the degree of deprotonation of arsenic acid) and the absolute concentrations of the phosphate ions influence the pH dependence of the retention times. In a phosphate buffer solution of pH 8 the concentration ratio $HPO_4^{2-}/H_2PO_4^-$ is 6.2 (apparent charge on phosphate, 1.86−) whereas at pH 5 this ratio is approximately 0.0062 (apparent charge, 1.00−). Thus at pH 8 the concentration ratio $HPO_4^{2-}/H_2PO_4^-$ is 1000 : 1, the actual concentration of HPO_4^{2-} being 144 times higher than at pH 5 at a constant total phosphate concentration of 0.03 mol dm^{-3}. The doubly charged HPO_4^{2-} anions are expected to compete effectively with the analyte anions for the positively charged groups at the surface of the stationary phase. The result of this competition is a decrease of retention time for arsenate with increasing pH. Arsenic acid is quite similar to phosphoric acid (Fig. 1). The charge on arsenate at pH 5 is 1−, at pH 8 almost 2−. Therefore, the change of charge from 1− at pH 5 to 2− at pH 8 and the competition from HPO_4^{2-} anions present at 40 times the concentration of $HAsO_4^{2-}$ are responsible for the observed pH dependence of the retention time of arsenate. The influence of the concentration of the phosphate anions on the retention times of arsenate was ascertained by chromatographing arsenate with a more dilute (0.006 mol dm^{-3}) phosphate buffer of pH 6. At this lower phosphate concentration the retention time was approximately four times the retention time with the 0.03 mol dm^{-3} mobile phase. Because both buffer solutions have the same pH, the ratio $H_2PO_4^-/HPO_4^{2-}$ must also be the same. However, the concentration of the HPO_4^{2-} in the 0.006 mol dm^{-3} phosphate buffer (3.5×10^{-4} mol dm^{-3}) is smaller than in the 0.03 mol dm^{-3} phosphate buffer (1.7×10^{-3} mol dm^{-3}). Because of the lower HPO_4^{2-} concentration the arsenate anions have a better chance to interact with the ammonium groups, causing longer retention times.

Optimal conditions for the separation of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid

An optimal chromatographic separation of the four arsenic compounds requires baseline separation at short retention times. The widths of the signals should be adjustable within limits to satisfy detector demands. The pH dependencies of the retention times (Figs 3, 4) suggest pH regions and mobile phases that are suitable for acceptable separations.

Acetate mobile phase

The optimal conditions with the 0.03 mol dm^{-3} acetate mobile phase are governed by the separation of arsenite and dimethylarsinic acid and by the length of retention time for arsenate. The most suitable pH range is 5–6. In this range arsenite and dimethylarsinic acid are well separated. Because the retention time for arsenate increases sharply between pH 5 and 6, the lower end of this pH range offers itself as an acceptable compromise. A separation of these four compounds is shown in Fig. 6. If a better separation of arsenite from dimethylarsinic acid is desired, a mobile phase with a pH closer to 6 can be used. Under these conditions the retention time for arsenate may increase to 14 min. Arsenate is always well separated from the other three arsenic compounds. However, the retention time of arsenate was sensitive to the conditioning time for the column, an observation not made for the other three arsenic compounds.

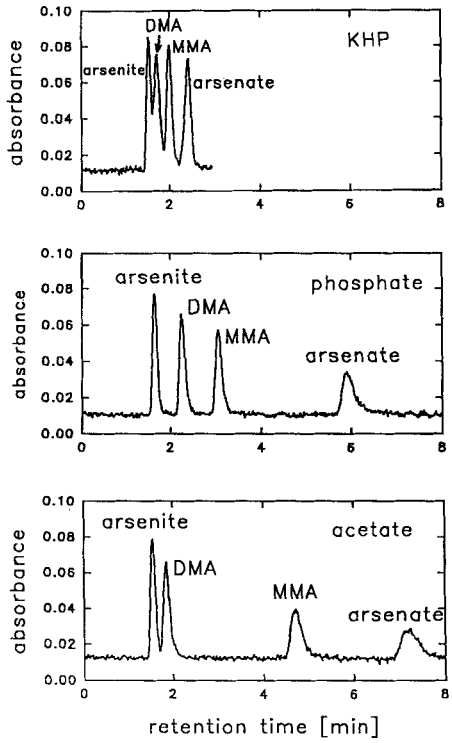

Figure 6 Separation of a mixture (100 µl) of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid (5 µg arsenic each) on a PRP-X100 anion-exchange column with a 0.006 mol dm^{-3} aqueous potassium hydrogen phthalate of pH 4, with a 0.03 mol dm^{-3} aqueous phosphate buffer of pH 6, and with a 0.03 mol dm^{-3} aqueous acetate buffer of pH 5 as mobile phase at a flow rate of 1.5 cm^3 min^{-1} (detector: FAA).

Phosphate mobile phase

Among the phosphate buffers the solution with pH 7 is least suited for the separation, because dimethylarsinic acid and methylarsonic acid have almost the same retention time of approximately 130 s. Separations of all four arsenic compounds are possible at pH 8 and between pH 5 and 6.5. However, with decreasing pH of the mobile phase, the retention time for arsenate increases from 260 s at pH 8 to 400 s at pH 5. A separation of the four arsenic compounds with a 0.03 mol dm^{-3} phosphate mobile phase at the optimal pH of 6 is shown in Fig. 6. A good separation with a shorter retention time for arsenate is also achievable at pH 8. A phosphate buffer of pH 6.2 was used by Chana and Smith[10] to separate these arsenic compounds on an Ionosphere anion-exchange column.

KHP mobile phase

Among the mobile phases investigated, KHP solutions produce the shortest retention times for all four arsenic compounds. To achieve usable retention times, the concentration of the KHP solutions had to be reduced to 0.006 mol dm^{-3}. The other two mobile phases were used as 0.03 mol dm^{-3} solutions. With 0.006 mol dm^{-3} KHP solutions the best separation is not a baseline separation, but has the advantage of requiring only 3 min. The largest differences in retention times between arsenite and methylarsonic/dimethylarsinic acid (18, 14 s) and methylarsonic acid and arsenate (~50 s) are achievable at pH 7. However, at this pH the two methylated compounds cannot be separated. If either methylarsonic acid or dimethylarsinic acid is not present in a sample, the KHP mobile phase is the best system for the separation of the three remaining arsenic compounds.

Experiments with 0.004 and 0.002 mol dm^{-3} KHP solutions of pH 4 as mobile phases showed that the separation of methylarsonic acid from dimethylarsinic acid and arsenate improves to baseline. However, arsenite and dimethyldarsinic acid are still not baseline-separated.

Which of the mobile phases can or should be used must be decided on the basis of the compatibility with the samples to be analyzed and the detector to be employed. To obtain low detection limits, the mobile phase must not interfere with the operation of the detector.

Compatibility of the mobile phases with detectors

Because compounds of arsenic and other elements of environmental importance are present in most samples at concentrations much lower than the organic and inorganic materials composing the matrix, 'general' detectors (conductivity, differential refractive index, UV–visible spectrometry) are often not useful in work with trace element compounds. This undesirable situation is largely remedied by element-specific detectors, which respond only to one or more elements and ideally are not influenced by matrix compounds.[11] Four major types of instruments have been applied most frequently as arsenic-specific detectors: flame atomic absorption spectrometers, graphite furnace atomic absorption spectrometers, inductively coupled plasma–mass spectrometers, and inductively coupled plasma–atomic emission spectrometers. The detection limits for arsenic achievable with GF AA and ICP detectors are much lower than with the FAA detector. However, at arsenic concentrations of

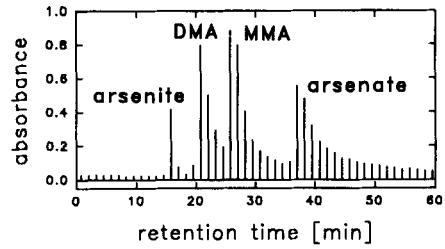

Figure 7 Separation of a mixture (100 µl) of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid (50 ng arsenic for arsenite, 300 ng arsenic for the other compounds) on a PRP-X100 anion-exchange column with a 0.005 mol dm^{-3} aqueous nickel sulfate solution of pH 6.3 as mobile phase at a flow rate of 0.15 cm^3 min^{-1} with GF AA detection.

10 mg dm^{-3} and higher, the FAA spectrometer is an excellent arsenic-specific detector. Many samples, however, have lower arsenic concentrations and, therefore, must be analyzed for arsenic compounds with GF AA or ICP detectors.

Because the GF AA spectrometer is the most widely used element-specific detector, the conditions used for the separation of arsenic compounds with the FAA detector were scrutinized for their applicability to systems with GF AA detection. To quantify the chromatograms obtained with GF AA detectors, a chromatographic band should be defined by a reasonable number of signals (not less than three) (Fig. 7). This number of signals (time between signals ≈1 min) can be obtained only when the chromatographic band is rather wide. Signal clusters of the required width can be generated by reducing the flow rate. To maintain good separation of the compounds under these conditions, the signal maxima must be as far removed from each other as possible without making the total analysis time too long. Chromatographing the four arsenic compounds under the conditions established as optimal for FAA detection but at lower flow rates brought the results presented below.

Potassium hydrogen phthalate mobile phase

Although the separation of the four arsenic compounds with 0.006 mol dm^{-3} aqueous KHP solution is sufficient for their identification with the FAA detector (Fig. 6), the retention times are too close to each other for the low flow rate (0.2 cm^3 min^{-1}) required by the GF AA detector. Only arsenite can be identified by a single signal at the solvent front. The signals from the other three compounds merge to a broad signal cluster characterized by pronounced tailing towards longer retention times (Fig. 8). Therefore, the KHP mobile phase is not usable with this HPLC–GF AA system.

Phosphate mobile phases

The FAA results indicate that the phosphate mobile phase of pH 6 is best suited for the separation of all four arsenic compounds in a reasonable time (Fig. 6). When the separation was carried out with GF AA detection at a flow rate of 0.2 cm^3 min^{-1}, signal clusters for the four compounds were present in the chromatogram (Fig. 8). The retention times were much longer at this low flow rate and the chromatographic bands became wider, as desired. The longer the retention time, the broader the bands became, producing the number of signals needed for quantification. Only arsenite, which migrates with the solvent front, was eluted in a rather narrow band that gave only one GF AA signal. Under these conditions, arsenite could be missed by the GF AA detector. The phosphate mobile phase

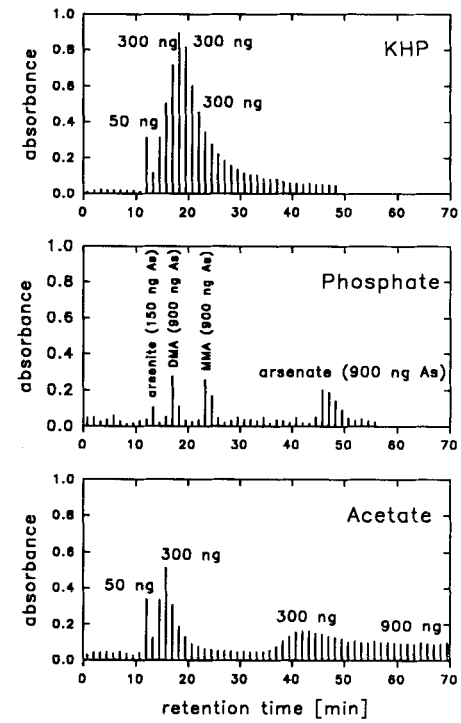

Figure 8 Separation of a mixture (100 µl) of arsenite (50, 150 ng arsenic), arsenate (300, 900 ng arsenic), methylarsonic acid (300, 900 ng arsenic), and dimethylarsinic acid (300, 900 ng arsenic) on a PRP-X100 anion-exchange column with 0.006 mol dm^{-3} aqueous potassium hydrogen phthalate of pH 4, 0.03 mol dm^{-3} phosphate buffer of pH 6, and with 0.03 mol dm^{-3} aqueous acetate buffer of pH 5 as mobile phase at a flow rate of 0.2 cm^3 min^{-1} and GF AA detection.

can be used to identify and quantify the four arsenic compounds. However, phosphate buffers are known to depress the arsenic signals obtained with GF AA.[12] This signal depression increases detection limits and is disadvantageous when samples with low concentrations of arsenic must be analyzed. Based on the signals in Fig. 8, the minimal amount of each arsenic compound that might be detectable is approximately 100 ng (arsenic) (1 mg dm^{-3}/100 µl HPLC injection, 20 µl GF AA). Phosphate-free mobile phases that do not depress the arsenic signals are desirable. Replacement of phosphate by another anion that allows similar separation of all four compounds with less severe signal suppresion would be advantageous.

Acetate mobile phase

A separation with the acetate mobile phase and GF AA detection is achievable (Fig. 8) at the flow rate of 0.2 cm^3 min^{-1}. Arsenite and dimethylarsinic acid are well separated and produce intense signals. Methylarsonic acid, with a retention time of 45 min, produces a broad signal cluster (width at baseline 15 min) that is still easily recognizable. The arsenate band is very broad and produces only a slightly elevated 'background'. The minimal amounts of arsenite and dimethylarsinic acid that are detectable are approximately 10 ng (0.1 mg dm^{-3}) (as arsenic). The acetate mobile phase can be used to identify much smaller amounts (approx one-tenth) of these two arsenic compounds than the phosphate mobile phase. Because of the broadness of the signal cluster for methylarsonic acid, 100 ng (as arsenic) must be present to be detectable, the same amount as is needed for the phosphate system. Arsenate must be present in amounts of at least a few micrograms. When arsenate must be detected, the phosphate mobile phase performs much better than the acetate solution.

Sodium sulfate mobile phase

Solutions of sodium sulfate in distilled water (0.006 mol dm^{-3}, not buffered, pH 5.9) as the mobile phase permitted baseline separation of arsenite, arsenate, dimethylarsinic acid and methylarsonic acid with FAA detection (Fig. 9). The elution sequence was the same as with the phosphate mobile phase. Sulfate anions—just like hydrogen phosphate anions—play an important role in the separation of the arsenic compounds. The retention times for methylarsonic acid and arsenate were much shorter with the

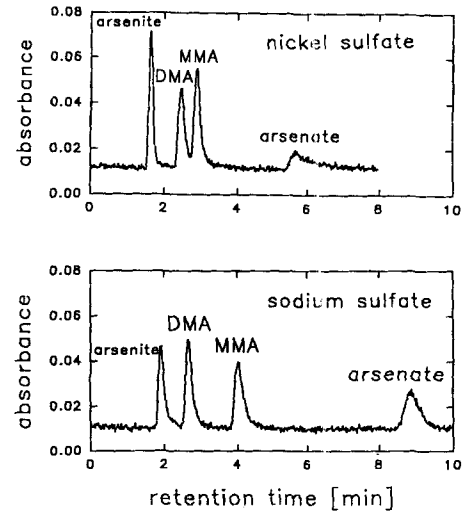

Figure 9 Separation of a mixture (100 µl) of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid (5 µg arsenic each) on a PRP-X100 anion-exchange column with a 0.006 mol dm^{-3} aqueous nickel sulfate solution of pH 6.3, and with a 0.006 mol dm^{-3} aqueous sodium sulfate solution of pH 5.9 as mobile phase at a flow rate of 1.5 cm^3 min^{-1} (detector: FAA).

0.006 mol dm^{-3} sulfate solution (3.8, 7.6 min) than with the 0.006 mol dm^{-3} phosphate solution (5.8, 23 min) (both solutions at pH 6). This decrease in retention times may be explained by the higher apparent charge of 2− in the case of sulfate, compared with an apparent charge of 1.06− on the phosphate anion at pH 6. Whereas the retention times of dimethylarsinic acid, methylarsonic acid and arsenate are smaller with the sulfate mobile phase (0.006 mol dm^{-3}), the retention time of arsenite increases from 97 to 104 s. One possible explanation may be that the higher ionic strength in the sulfate mobile phase decreases the pK value of arsenous acid.[13] As a consequence, deprotonation of arsenous acid in the higher-ionic-strength medium begins at lower pH than in the low-ionic-strength medium, with a concomitant increase of the retention time. The results of these experiments indicate that a sodium sulfate solution as mobile phase is suitable for the separation of arsenite, arsenate, dimethylarsinic acid and methylarsonic acid. With the GF AA detector arsenite, dimethylarsinic acid and methylarsonic acid are easily identifiable. The signal cluster for methylarsonic acid tails badly and the cluster for arsenate disappears in the background. Thus, the sodium sulfate mobile phase is not suitable for the detection of low concentrations of arsenate. The signal intensities

for the other three compounds are higher with sodium sulfate than with phosphate solutions (Figs 8, 10). Sodium sulfate also reduces the GF AA arsenic signals, although to a much lesser extent than phosphate. For instance, sodium sulfate at a concentration of 772 mg dm^{-3} reduces the arsenic signal of a 100 µg dm^{-3} arsenic (as arsenite or arsenate) solution by 5% at an ashing temperature of 900 °C, whereas phosphate achieves the same reduction at the much lower concentration of 41 mg dm^{-3}.[12] Therefore, arsenite, methylarsonic acid and dimethylarsinic acid are detectable with the sodium sulfate mobile phase, when at least 10 ng arsenic as arsenite, 100 ng arsenic as methylarsonic acid, and dimethylarsinic acid are present in the 100 µl injected.

Nickel sulfate mobile phase

Addition of nickel salts to samples in which arsenic should be determined by GF AA is known to increase signal intensities. Therefore, the use of nickel sulfate as a component of the mobile phase could lead to more intense signals, because the presence of nickel might counteract the signal depression by sulfate. Indeed, 0.006 mol dm^{-3} nickel sulfate solution allowed the separation of arsenite, dimethylarsinic acid, methylarsonic acid and arsenate (FAA detection) in an even shorter time than sodium sulfate does (Fig. 9). Disadvantageously, the arsenate peak was broad and tailed, whereas the tailing phenomenon was not observed with either the phosphate mobile phase or the sodium sulfate mobile phase (FAA detection). For the separation and detection of the arsenic compounds with nickel sulfate as mobile phase and GF AA detection, the flow rate was decreased to 0.15 cm^3 min^{-1}. The chromatogram obtained is shown in Fig. 7. Whereas the peak clusters for arsenite, methylarsonic acid and dimethylarsinic acid are nearly symmetric, the signal cluster for arsenate shows considerably tailing. The four arsenic compounds are detectable when at least 10 ng arsenic from each compound is present.

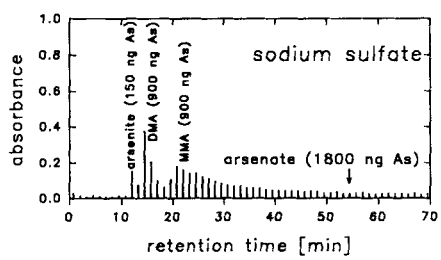

Figure 10 Separation of a mixture (100 µl) of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid on a PRP-X100 anion-exchange column with a 0.006 mol dm^{-3} sodium sulfate of pH 5.9 as mobile phase at a flow rate of 0.2 cm^3 min^{-1} and GF AA detection.

CONCLUSION

Investigation of the retention behavior of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid on the PRP-X100 anion-exchange column, influenced by the pH, the concentration, and the nature of buffer solutions, showed that optimal separation of these four arsenic compounds (FFA detection; flow rate 1.5 cm^3 min^{-1}) is possible with 0.03 mol dm^{-3} phosphate buffer at pH 6 and 8, with 0.03 mol dm^{-3} acetate buffer at pH between 5 and 6, and with 0.006 mol dm^{-3} potassium hydrogen phthalate at pH 4 (no baseline separation of arsenite and dimethylarsinic acid). These results clearly indicate that the optimal separation of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid with buffer solutions is not only determined by the pH of the mobile phase, but also by the anions introduced by the mobile phase and their absolute concentration. At concentrations greater than 10 mg dm^{-3} (100 µl loop) arsenic per compound, FAA is the optimal detector for the identification of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid using either the phosphate or the acetate buffer. Below 10 mg dm^{-3} arsenic, other detectors must be used to identify arsenic compounds. GF AA detectors coupled to chromatographs offer much lower detection limits for arsenic (~1 mg dm^{-3}) but require much lower flow rates (~0.2 cm^3 min^{-1}). Among the optimized FAA mobile phases, only the phosphate buffer of pH 6 allowed the identification of all four arsenic compounds under the conditions required for GF AA detection. Phosphate is known to depress the arsenic signal in GF AA detection. A 0.006 mol dm^{-3} (FAA detection; flow rate 1.5 cm^3 min^{-1}) and 0.005 mol dm^{-3} (GF AA detection; flow rate 0.15 cm^3 min^{-1}) nickel sulfate solution permitted good separation of all four arsenic compounds with both systems. The nickel sulfate mobile phase supplying a matrix modifier for the determination of arsenic is therefore best suited to achieve low detection limits (~10 ng absolute, 0.1 mg dm^{-3}, 100 µl loop, per arsenic compound). This detection limit of 10 ng arsenic

for the HPLC–GF AA system is approximately 100 times higher than the detection limits of 0.1 ng arsenic achievable by injection of an aqueous solution of arsenate or arsenite with nickel sulfate as matrix modifier. The increase in detection limit is caused by the dilution of the 100 µl of the chromatographed solution during the migration through the column with concomitant spreading into a chromatographic band with a baseline width of several minutes.

All these results were obtained with matrix-free, distilled-water solutions of arsenic compounds. With matrix-laden solutions, such as extracts from biological samples, unbuffered mobile phases (e.g. nickel sulfate) might not be usable, because matrix components could appreciably change the pH of the solutions. In these cases, the buffered phosphate mobile phases might be a better choice. High concentrations of various anions might also change the retention behavior of the arsenic compounds. Organic compounds may not seriously interfere with the anion-exchange separation of the arsenic compounds. The results presented in this paper serve as the basis for the development of conditions for the separation of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid in complex matrices. Should separations not be achievable under the conditions reported in this paper, the matrix-laden solution to be chromatographed must be purified.[14]

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