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Journal of Chromatography A, 730 (1996) 219–229

JOURNAL OF CHROMATOGRAPHY A

Retention behavior of arsenobetaine, arsenocholine, trimethylarsine oxide and tetramethylarsonium iodide on a styrene–divinylbenzene column with benzenesulfonates as ion-pairing reagents

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Abstract

The pH-dependent retention behavior of arsenobetaine, arsenocholine, trimethylarsine oxide, tetramethylarsonium iodide (cationic arsenic compounds), arsenite, arsenate, methylarsonic acid, and dimethylarsinic acid (anionic arsenic compounds) was studied on a Hamilton PRP-1 reversed-phase column (250×4.1 mm I.D.) with 10 m*M* aqueous solutions of benzensulfonic acids ($X\text{-}C_6H_4SO_3^-$; X=H, 4-HO, 3-CO_2H; 4-HO-3-$HO_2C\text{-}C_6H_3SO_3^-$) as ion-pairing reagents in the pH range 2–5 using flame atomic absorption spectrometry as the arsenic-specific detector. The dependencies of the k'-values of the 'cationic' arsenic compounds was rationalized on the basis of the protonation/deprotonation behavior of the arsenic compounds and of the four benzenesulfonates. The results provided evidence for the formation of a cationic species from trimethylarsine oxide below pH 3. Benzenesulfonate is the most hydrophobic ion-pairing reagent causing strong retention of the cationic arsenic compounds and consequently impeding their rapid separation. With the less hydrophobic, substituted benzenesulfonates the cationic arsenic compounds had retention times not exceeding 6 min. At a flow-rate of 1.5 $cm^3\ min^{-1}$ 10 m*M* aqueous 3-carboxy-4-hydroxybenzenesulfonate solution adjusted to pH 3.5 allowed the separation of arsenate, methylarsonic acid, arsenobetaine, trimethylarsine oxide, the tetramethylarsonium ion, and arsenocholine within 3 min. Dimethylarsinic acid coelutes with arsenobetaine at pH 3.5, but can be separated from arsenobetaine with the same mobile phase at pH 2.5. At pH 2.5 the signals for trimethylarsine oxide, the tetramethylarsonium ion, and arsenocholine are too broad to be useful for quantification. Arsenite and methylarsonic acid cannot be separated under these conditions.

Keywords: pH optimization; Retention behaviour; Optimization; Arsenic compounds; Benzenesulfonates; Inorganic ions

1. Introduction

Arsenic compounds occur in organisms, such as marine algae, bivalves, crustaceans, gastropods, cephalopods, echinoderms and marine fishes [1], and in the abiotic environment [2]. Arsenite and arsenate are found in the environment because of weathering of primary arsenic minerals and anthropogenic emissions. Biologically mediated methylation reactions occurring in terrestrial and marine organisms convert arsenite and arsenate to methylated arsenic compounds, such as methylarsonic acid and dimethylarsinic acid. More complex arsenic compounds (arsenobetaine, trimethylarsine oxide, arsenocholine, tetramethylarsonium salts, arsenosugars) were identified in marine organisms. In addition, arsenobetaine has also been found in terrestrial biota [3].

Several techniques are available for the determi-

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0021-9673/96/$15.00
SSDI 0021-9673(95)01187-0

nation of concentrations of total arsenic [4,5]. However, once the total concentration of arsenic is known, the arsenic compounds must be identified because different species follow different metabolic pathways and generally have different toxicities [6]. Consequently, the arsenic compounds present in environmental and biological samples must be known before the degree of environmental pollution and the toxicological properties of arsenic-containing food items can be established.

Hyphenated techniques are very useful for the identification of arsenic compounds. These techniques couple a separation method with element-specific detectors such as flame atomic absorption spectrometers (FAAS), graphite furnace atomic absorption spectrometers (GFAAS), inductively coupled argon plasma mass spectrometers (ICP-MS), or inductively coupled plasma atomic emission spectrometers (ICP-AES) [5]. Among the separation techniques, high-performance liquid chromatography (HPLC) has been applied most frequently for the identification of arsenic compounds [7]. In HPLC, arsenic species can be separated by anion-exchange, cation-exchange, or reversed-phase columns. The choice is determined by the compounds to be separated and the detectors to be used. Recently, we published results of a systematic investigation of the retention behavior of arsenous acid, arsenic acid, methylarsonic acid, and dimethylarsinic acid utilizing anion-exchange chromatography (a Hamilton PRP-X100 anion-exchange column) with FAAS and GFAAS as element-specific detectors [8].

The arsenic compound most frequently found in marine animals is arsenobetaine. Tetramethylarsonium salts, trimethylarsine oxide, and arsenocholine may also be present [9]. The separation of these 'cationic' arsenic compounds requires a cation-exchange or a reversed-phase separation technique. Few papers were found in the literature reporting a separation method for arsenobetaine, arsenocholine, the tetramethylarsonium cation, and trimethylarsine oxide by cation-exchange chromatography [10–12]. Gel chromatography was also applied to separate these arsenic compounds [12,13]. However, details about the conditions for the separation were not reported.

Ion-pair chromatography using heptanesulfonic acid [14–16] or dodecylbenzenesulfonic acid [14] was used to separate arsenobetaine from arsenocholine. Arsenobetaine, arsenocholine, and tetramethylarsonium iodide were separated with heptanesulfonic acid [17] or 2,4,6-trinitro-5-hydroxybenzenesulfonic acid [18]. Shibata and Morita [13] reported the reversed-phase separation of arsenobetaine, arsenocholine, tetramethylarsonium iodide, and trimethylarsine oxide with butanesulfonic acid as the ion-pairing reagent. We investigated the pH-dependent retention behavior of arsenobetaine, arsenocholine, trimethylarsine oxide, tetramethylarsonium iodide, arsenite, arsenate, methylarsonic acid, and dimethylarsinic acid with four benzenesulfonic acids as ion-pairing reagents on a Hamilton PRP-1 column.

2. Experimental

2.1. Chemicals

$NaAsO_2$, $Na_2HAsO_4 \cdot 7H_2O$, NaOH, NaCl, and 3-carboxy-4-hydroxybenzenesulfonic acid dihydrate were of analytical grade and purchased from Merck (Darmstadt, Germany). Reagent-grade benzenesulfonic acid (~92%) was also purchased from Merck. The aqueous 4-hydroxybenzenesulfonic acid solution (65%) and sodium heptanesulfonate monohydrate (>99%) were obtained from Fluka (Buchs, Switzerland) and sodium 3-carboxybenzenesulfonate (>97%) from Aldrich (Milwaukee, MI, USA). The pK_a of the carboxylic acid group in 3-carboxybenzenesulfonic acid was determined by titration of a 0.01 *M* aqueous solution of the acid with 0.01 *M* sodium hydroxide. The pH during the titration was measured with a pH meter (Orion SA 720 pH meter, Boston, MA, USA). The pK_a was found to be 3.8 (pK_a of benzoic acid 4.2). Methylarsonic acid was recrystallized from methanol (m.p. 156°C). Dimethylarsinic acid (m.p. 190°C) was dried over phosphorus pentoxide. Arsenobetaine bromide [trimethyl(2-carboxy-methyl)arsonium bromide] was synthesized by the method described by McShane [19]. Purification by recrystallisation from ethanol yielded white crystals with a melting point of 225°C (lit. [19] 227°C). Arsenocholine bromide was prepared according to a published procedure [20]. The product was recrystallized from dry acetonitrile. The

white, hygroscopic crystals were isolated in a dry box and stored in a vacuum desiccator over phosphorus pentoxide. Trimethylarsine oxide was prepared by oxidation of trimethylarsine dissolved in diethyl ether with hydrogen peroxide (30%) [21]. The oxide was purified by sublimation [160°C, 20 Torr (ca. 2.7 kPa), m.p. 188°C]. Tetramethylarsonium iodide was recrystallized from methanol. Triply distilled water (quartz still, Destamat, Heraeus, Kleinostheim, Germany) was used to prepare all solutions.

2.2. Solutions

Solutions of the arsenic compounds containing 50 mg of arsenic per dm^{-3} (range 0.57–0.74 m*M* As) were prepared by dissolving 52.04 mg $Na_2HAsO_4 \cdot 7H_2O$, 22.58 mg $NaAsO_2$, 22.34 mg methylarsonic acid, 25.50 mg dimethylarsinic acid, 43.61 mg arsenobetaine bromide, or 37.26 mg tetramethylarsonium iodide to 250 cm^3. Because arsenocholine and trimethylarsine oxide are very hygroscopic, 12.5 cm^3 of 1000 mg As dm^{-3} solutions of these compounds (21.14 mg trimethylarsine oxide, 37.94 mg arsenocholine bromide) were diluted to 250 cm^3.

Solutions (10 m*M*) of the ion-pairing reagents were prepared by dissolving 2.542 g 3-carboxy-4-hydroxybenzenesulfonic acid, 2.680 g of the 65% aqueous solution of 4-hydroxybenzenesulfonic acid, or 2.240 g sodium 3-carboxybenzenesulfonate to 1 dm^3 with triply distilled water. The pH was adjusted with 2 *M* NaOH to 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5.0 (with sodium 3-carboxybenzenesulfonate only pH 3.0, 3.5, 4.0, 4.5, or 5.0). Solutions (~10 m*M*) of benzenesulfonic acid of pH 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.7, or 7.7 were prepared by slurrying 1.582 g in 1 dm^3 of triply distilled water and stirring the slurry for 2 h. The cloudy solution was filtered through a 0.65-μm cellulose nitrate filter. The residue on the filter was dried. The mass of a dry single filter was subtracted from the dry filter through which the solution had been passed. With consideration of the benzenesulfonic acid recovered (29.04 mg) the acid concentration in the mobile phase was 9.8 m*M*. The clear ~10 m*M* benzenesulfonic acid solution was then adjusted to the required pH by dropwise addition of 2 *M* NaOH. Aqueous solutions of sodium heptanesulfonate (10 m*M*) of pH 2.9, 3.8, 4.5, 5.4, 6.6, or 8.2 were prepared by dissolving 2.203 g sodium heptanesulfonate with triply distilled water to 1 dm^3. The pH was adjusted by dropwise addition of conc. hydrochloric acid or 2 *M* NaOH.

2.3. Instrumentation

The HPLC system consisted of a Milton Roy CM-4000 multiple solvent delivery unit and a PRP-1 column (Hamilton, Reno, NV, USA; 250×4.1 mm I.D.; spherical, 10-μm particles of a styrene–divinylbenzene copolymer). A 100-μl loop was used in conjunction with a Rheodyne 6-port injection valve. A guard cartridge (Hamilton) filled with the same stationary phase protected the analytical column. A Z-6100 flame atomic absorption spectrophotometer (Hitachi, Tokyo, Japan) served to detect the arsenic compounds in the column effluent. Details about the HPLC–FAAS system were published earlier [8].

The column was thermostated at 25°C and equilibrated by passing at least 100 cm^3 (flow-rate 1 $cm^3\ min^{-1}$) of the mobile phase through the column before injection of the arsenic compounds. Aliquots (100 μl) of the solutions of the arsenic standards containing 5 μg arsenic 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 (R.S.D. <2%).

The dead volume was determined by injection (100 μl) of 1 mg Na dm^{-3} aqueous sodium chloride solution with distilled water serving as the mobile phase and on-line FAAS detection of sodium at 589 nm to be 1.4 cm^3 corresponding to a dead time of 56 s at a flow-rate of 1.5 $cm^3\ min^{-1}$.

3. Results and discussion

Arsenic compounds (trimethylarsine oxide, tetramethylarsonium iodide, arsenocholine, arsenobetaine) that are or can be present as cations in solution have been separated on reversed-phase columns with sulfonic acids as ion-pairing reagents. The sulfonic acid groups in these reagents are ionized throughout the pH range (1 to 13) accessible with the PRP-based columns. The sulfonate anions are needed for the formation of ion-pairs. Additional

functional groups (hydroxyl, carboxyl) that can be present in anionic or neutral form in the sulfonic acid molecule could increase or decrease the hydrophobic character of the ion-pairs. Because the pK_a-values of the carboxylic groups are in the pH range studied, the k'-values of the arsenic compounds can be changed by alteration of the pH of the mobile phase. Optimal conditions for the separations can then be chosen.

3.1. The solution chemistry of cationic arsenic species

Arsenobetaine, arsenocholine, the tetramethylarsonium cation, and trimethylarsine oxide all contain the $(CH_3)_3As$ moiety. The degree to which the ionizable groups are dissociated influences the charge on the species and consequently their separation. Arsenocholine and the tetramethylarsonium ion are positively charged irrespective of pH. Arsenobetaine with a pK_a of 2.18 [22] is a zwitterion above pH 4.5 and a cation below pH 1. Between pH 1 and 4.5 both species are present in varying ratios (Fig. 1).

The behavior of trimethylarsine oxide in the pH range 2–5 is less well known. Because trimethylarsine oxide is very hygroscopic, the As=O group will interact with water. On dissolution of trimethylarsine oxide, a water molecule can be hydrogen-bonded to the oxygen atom of the As=O group (Eq. 1), or can react to form trimethyldihydroxyarsane (Eq. 2).

$$R_3AsO + H_2O \rightleftharpoons R_3AsO \cdots HOH \quad (1)$$

$$R_3AsO + H_2O \rightleftharpoons R_3As(OH)_2 \quad (2)$$

The dihydroxy compound could be present as a neutral molecule or as a trimethylhydroxyarsonium hydroxide, in which one OH-group serves as the anion (Eq. 3):

$$R_3As(OH)_2 \rightleftharpoons [R_3As-OH]^+OH^- \quad (3)$$

When an aqueous solution of trimethylarsine oxide is acidified, for instance with a hydrohalic acid, HX, a neutralization reaction could form $[R_3As\text{-}OH]^+ X^-$. The anion X^- may be covalently bound to the arsenic atom yielding a trimethylhalohydroxyarsane, which may be in equilibrium with the trimethyl(hydroxy)arsonium cation (Eq. 4). The cationic species $[R_3As\text{-}X]^+OH^-$ cannot be present at a concentration $>1\times10^{-10}$ M in a solution of pH 4.

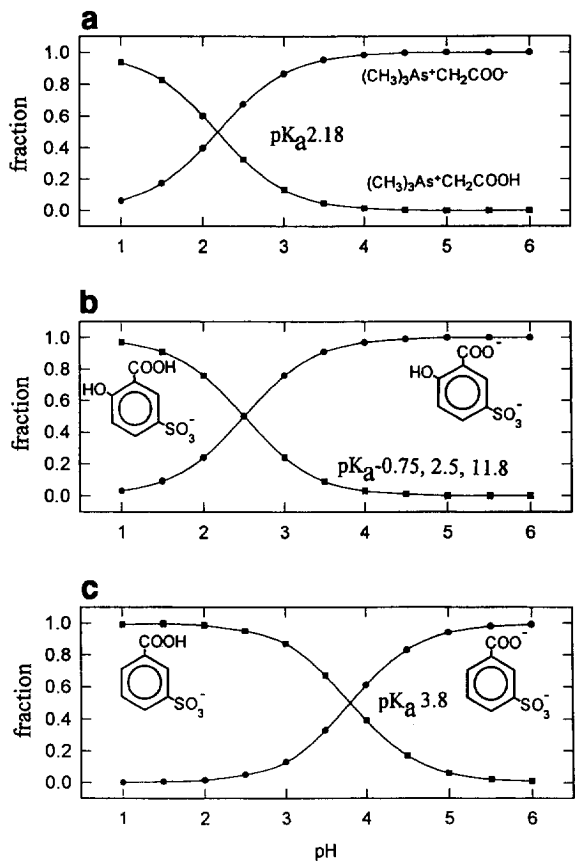

Fig. 1. Species distribution diagram for (a) arsenobetaine (pK_a [22]), (b) 3-carboxy-4-hydroxybenzenesulfonic acid (pK_a [23]), and (c) 3-carboxybenzenesulfonic acid in the pH range 1 to 6.

$$[R_3As-OH]^+X^- \rightleftharpoons R_3As(OH)(X) \quad (4)$$

Neutral $R_3As(OH)(X)$ may react further with HX (Eq. 5):

$$R_3As(OH)(X) + HX \rightleftharpoons R_3AsX_2 + H_2O \quad (5)$$

Again, an equilibrium may be set up between the neutral trimethyldihaloarsane and the corresponding arsonium halide (Eq. 6):

$$R_3AsX_2 \rightleftharpoons [R_3AsX]^+X^- \quad (6)$$

The nature of the species formed upon dissolution of trimethylarsine oxide will influence the ion-pairing reaction and thus the retention on the reversed-phase column. Conversely, the retention behavior will provide information about the 'molecular status' of trimethylarsine oxide.

3.2. Retention of arsenobetaine and trimethylarsine oxide with heptanesulfonate as ion-pairing reagent

To obtain information about cationic species that may be formed from trimethylarsine oxide, aqueous solutions of this compound were chromatographed with mobile phases 10 m*M* with respect to sodium heptanesulfonate in the pH range 3–8. Arsenobetaine, which exists as a mixture of the zwitterion and the arsonium cation in the pH range 1–4.5 (Fig. 1), was chromatographed to establish the behavior of a compound that changes from positively charged to uncharged. Fig. 2 shows the dependence of the k'-values of arsenobetaine and trimethylarsine oxide on the pH of the mobile phase using the PRP-1 reversed-phase column and FAAS detection at 193.7 nm. The k' of trimethylarsine oxide (TMAO) decreases from 7.43 at pH 4.5 to 1.35 at pH 5.4. Further increase of pH does not change the retention appreciably (Fig. 2). The k' for arsenobetaine at pH 2.9 (~85% present as zwitterion, apparent charge +0.15) is 9.16 and decreases to 0.93 at pH 4.5 (Fig. 2). This decrease in k' with increasing pH is attributable to the decrease in the concentration of the arsonium species and the increase in the concentration of the zwitterion and thus to the decrease of the apparent charge on arsenobetaine in this pH range. At pH-values above 4.5 the zwitterion is the only species of arsenobetaine in solution which is not capable of forming ion-pairs with heptanesulfonate. Therefore, the k' of arsenobetaine remains at approximately 0.8 between pH 4.5 and 8.2 (Fig. 2). That arsenobetaine is not eluted with the dead volume is attributable to hydrophobic interactions between the arsenobetaine molecules and the organic backbone of the stationary phase.

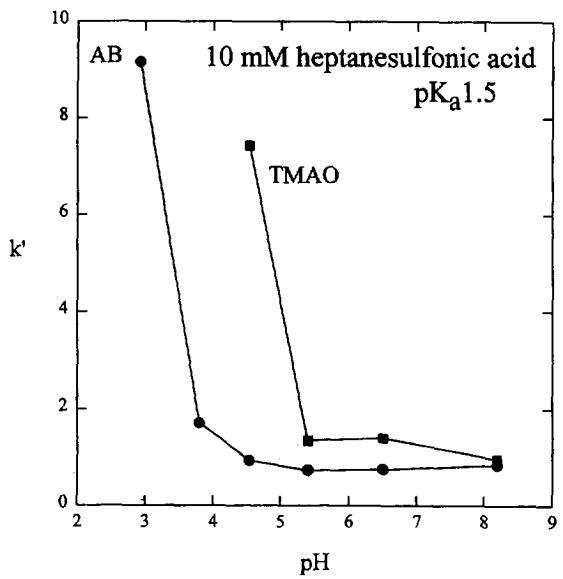

Fig. 2. Dependence of the k'-values of arsenobetaine (AB) and trimethylarsine oxide (TMAO) on pH with an aqueous 10 m*M* solution of 1-heptanesulfonic acid (pK_a ~1.5 [24]) as the mobile phase (column, PRP-1, 250×4.1 mm I.D.; flow-rate, 1.5 cm^3 min^{-1}; detector, FAAS at 193.7 nm; loop, 100 μl; 5 μg As of each compound injected separately).

If the decrease in apparent positive charge on trimethylarsine oxide in the pH range 4.5–5.4 is the cause for the observed decrease in the retention time, the equilibrium between the hydroxy-(trimethyl)arsonium heptanesulfonate and the (heptanesulfonato)hydroxy-(trimethyl)arsane must shift toward the uncharged species with increasing pH (Eq. 4, OH^- to be replaced by $C_7H_{15}SO_3^-$). If this assumption is correct, only the uncharged species exists at pH 5.4 that is expected on account of the hydrophobic heptyl group to have more affinity to the hydrophobic stationary phase than the arsenobetaine zwitterion. The k' of trimethylarsine oxide at pH 5.4 ($k'=1.35$) with a value approximately twice the k' for arsenobetaine ($k'=0.73$) supports this hypothesis.

3.3. Retention of tetramethylarsonium iodide and arsenocholine bromide with benzenesulfonates as ion-pairing reagents

Tetramethylarsonium iodide and arsenocholine bromide produce on dissolution in water arsonium cations that remain cations throughout the investigated pH range. Therefore, changes in k'-values

can only be caused by the ion-pairing reagents and the competition between the arsonium ions, H^+, and other cationic species (for instance Na^+ introduced through the pH adjustment with NaOH).

The observed changes of the k'-values in the pH range 2–5 are very similar for the two arsenic compounds chromatographed with the same benzenesulfonate as ion-pairing reagent (Fig. 3). Among the investigated ion-pairing reagents, arsenocholine ion-pairs generally have k'-values of 0.1 to 2.0 k'-units larger than the corresponding tetramethylarsonium ion-pairs (Fig. 3) suggesting that arsenocholine is more hydrophobic than the tetramethylarsonium ion.

Benzenesulfonate as ion-pairing reagent

The k'-values of the two arsonium cations increase monotonically by approximately 2.8 k'-units, when the mobile phase pH is changed from 2 to 4 (Fig. 3). Because the ionic state of neither the sulfonate nor the arsonium cations are influenced by the pH change, the trend in the retention times must be attributed to the changing adsorption of the ion-pairing reagent as a result of the replacement of H_3O^+ by the Na^+ introduced during pH adjustment. The k'-values for these two compounds with the other three benzenesulfonates (0.8 to 5.8) are much smaller than the corresponding k'-values obtained with benzenesulfonic acid (5.3 to 9.8) as ion-pairing reagent (Fig. 3). The rather large differences can be attributed to the lower hydrophobicity of the substituted benzenesulfonates (due to the hydroxy and/or the carboxy groups).

3-Carboxybenzenesulfonate as ion-pairing reagent

The k'-values of the tetramethylarsonium and arsenocholine cations decrease almost linearly by approximately 3.3 k'-units upon increase of the pH from 3 to 5. In this pH range the carboxy group (pK_a=3.8) changes from almost completely protonated at pH 3 to almost completely deprotonated at pH 5 (Fig. 1). Because the sulfonic acid group (pK_a=0.7) is completely dissociated in the pH range 2–5, the fraction of the dinegative molecule and consequently the average charge on the molecule increase with increasing pH. At pH 5 almost all molecules carry two negative charges causing the pairing ion and the ion-pair to be more hydrophilic

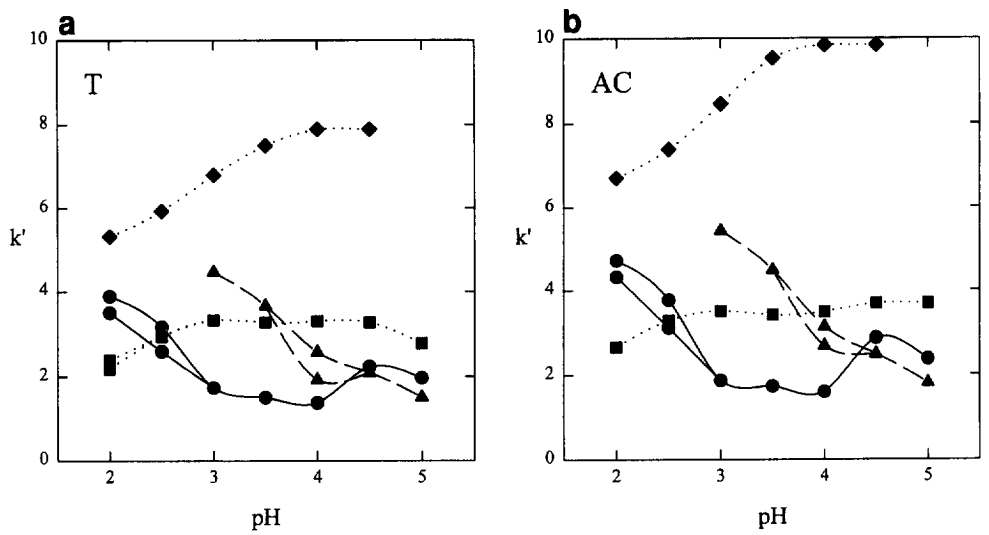

Fig. 3. Dependence of the k'-values of (a) tetramethylarsonium iodide (T) and (b) arsenocholine (AC) on the pH of the mobile phases and the ion-pairing reagent [10 mM aqueous solutions of benzenesulfonic acid (◇), 3-carboxy-4-hydroxybenzenesulfonic acid (●), 4-hydroxybenzenesulfonic acid (■), and 3-carboxybenzenesulfonic acid (▲)] in the pH range 2–5 (column, PRP-1, 250×4.1 mm I.D.; flow-rate, 1.5 $cm^3\ min^{-1}$; detector, FAAS at 193.7 nm; loop, 100 μl; 5 μg As of each compound injected separately).

and retained more weakly on the hydrophobic PRP-1 phase. At pH 4 arsenocholine and tetramethylarsonium produce two peaks each. The cause of this reproducible behavior is not understood at this time.

4-Hydroxybenzenesulfonic acid as ion-pairing reagent

The k'-values for the tetramethylarsonium ion and arsenocholine do not vary more than approximately 0.8 k'-units over the pH range 2–5. In 4-hydroxybenzenesulfonic acid the sulfonic acid group is completely dissociated and the phenolic OH-group remains undissociated over the pH-range investigated. Because the apparent and actual charges on the ions forming ion-pairs do not change, appreciable differences in k'-values are not expected and are not observed.

3-Carboxy-4-hydroxybenzenesulfonic acid

Between pH 2 and 5, the k'-values of arsenocholine and tetramethylarsonium iodide are very much alike. The k'-values of tetramethylarsonium are only 0.1 to 0.8 k'-units smaller than the corresponding values for arsenocholine. The k'-values decrease as the pH is increased from 2 to 4, a behavior attributable to the dissociation of the carboxylic group (pK_a=2.5, Fig. 1). Above pH 4 the retention increases again, the cause of which is presently not understood. Chromatograms obtained with the mobile phase at pH 2 and 2.5 (but not at pH 3 and higher pH-values) show, that the signals for arsenocholine and the tetramethylarsonium ion are split into two peaks each.

3.4. Retention of arsenobetaine with benzenesulfonates as ion-pairing reagents

The pH-dependent retention behavior of arsenobetaine with the four benzenesulfonates is shown in Fig. 4. The observed dependence of the k'-values of arsenobetaine on the pH of the mobile phases is markedly different from the retention behavior of arsenocholine and tetramethylarsonium iodide, because the carboxy group of arsenobetaine with a pK_a of 2.18 allows the compound to become completely zwitterionic above pH 4.5.

With benzenesulfonate as the most hydrophobic

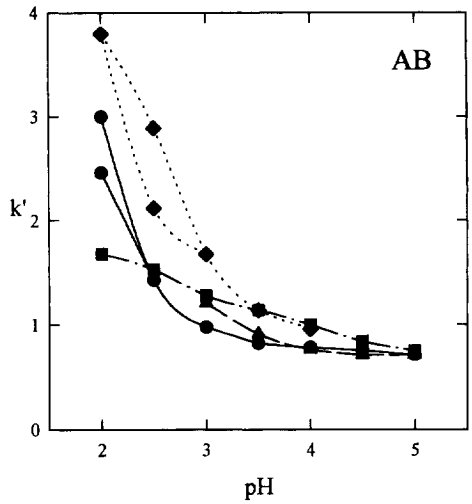

Fig. 4. Dependence of the k'-values of arsenobetaine (AB) on the pH of the mobile phases and the ion-pairing reagent [10 m*M* aqueous solutions of benzenesulfonic acid (◇), 3-carboxy-4-hydroxybenzenesulfonic acid (●), 4-hydroxybenzenesulfonic acid (■), and 3-carboxybenzenesulfonic acid (▲)] in the pH range 2–5 (column, PRP-1, 250×4.1 mm I.D.; flow-rate, 1.5 $cm^3\ min^{-1}$; detector, FAAS at 193.7 nm; loop, 100 μl; 5 μg As injected).

ion-pairing reagent among the investigated benzenesulfonates, the k' for arsenobetaine decreases from 3.8 at pH 2 to 1.0 at pH 4. At pH 2.5 arsenobetaine produces two peaks. 3-Carboxybenzenesulfonate as ion-pairing reagent produces k'-values that decrease from 1.2 at pH 3 to 0.8 at pH 4 and remain at approximately 0.7 upon further increase of the pH to 5. No double peaks were observed. With 4-hydroxybenzenesulfonic acid as ion-pairing reagent the k' of arsenobetaine decreases from 1.7 at pH 2 to 0.7 at pH 5. No double peaks were detected.

3-Carboxy-4-hydroxybenzenesulfonic acid as ion-pairing reagent

The general pH-dependent retention behavior of arsenobetaine with 3-carboxy-4-hydroxybenzenesulfonate is similar to the retention behavior observed with the benzenesulfonate mobile phase. The k'-values obtained with the substituted benzenesulfonates are 0.2 to 1.1 k'-units smaller than the values for benzenesulfonate-containing mobile phase. The

differences in the k'-values are caused by the higher hydrophobicity of benzenesulfonate. The k'-value for arsenobetaine decreases markedly from approximately 2.7 at pH 2 to 0.8 at pH 3.5 and remains unchanged upon further increase of the pH to 5. At pH 2 arsenobetaine produces two peaks.

3.5. Retention of trimethylarsine oxide with benzenesulfonates as ion-pairing reagents

Trimethylarsine oxide shows almost the same pH-dependence of the k'-values in the pH range 2–3 as arsenocholine and the tetramethylarsonium ion (Fig. 5 and Fig. 6). This similarity suggests that trimethylarsine oxide has a similar positive apparent charge as arsenocholine and the tetramethylarsonium ion. Presumably, trimethylarsine oxide has one positive charge in this pH range.

3-Carboxybenzenesulfonate as ion-pairing reagent produces k'-values for trimethylarsine oxide that decrease from 3.4 at pH 3 to 1.3 at pH 4 and down to 0.9 at pH 5. At pH 3.5 trimethylarsine oxide produced two peaks. With 4-hydroxybenzenesulfonate the k' of trimethylarsine oxide increases marginally from 1.9 at pH 2 to 2.0 at 3 and decreases to 1.1 at pH 5. At pH 3.5 the trimethylarsine oxide peak is split into two signals.

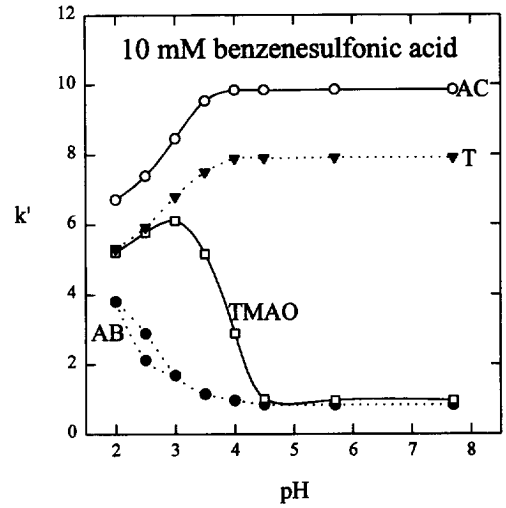

Fig. 6. Dependence of the k'-values of arsenobetaine (AB), trimethylarsine oxide (TMAO), tetramethylarsonium (T), and arsenocholine (AC) on the pH of 10 m*M* benzenesulfonic acid in the pH range 2–7.7 (column, PRP-1, 250×4.1 mm I.D.; flow-rate, 1.5 $cm^3\ min^{-1}$; detector, FAAS at 193.7 nm; loop, 100 μl; 5 μg As of each compound injected separately).

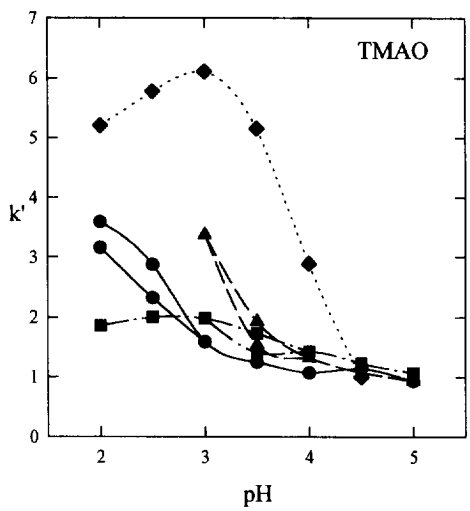

Fig. 5. Dependence of the k'-values of trimethylarsine oxide (TMAO) on the pH of the mobile phases and the ion-pairing reagent [10 m*M* aqueous solutions of benzenesulfonic acid (◇), 3-carboxy-4-hydroxybenzenesulfonic acid (●), 4-hydroxybenzenesulfonic acid (■), and 3-carboxybenzenesulfonic acid (▲)] in the pH range 2–5 (column, PRP-1, 250×4.1 mm I.D.; flow-rate, 1.5 $cm^3\ min^{-1}$; detector, FAAS at 193.7 nm; loop, 100 μl; 5 μg As injected).

Benzenesulfonic acid

Trimethylarsine oxide and the tetramethylarsonium ion have almost identical k'-values at pH 2 (5.21, 5.32) and pH 2.5 (5.78, 5.93) (Fig. 6). However, upon further increase of pH the k'-value of trimethylarsine oxide begins to decrease reaching 0.96 at pH 4.5. In the pH range 3–4.5 the retention behavior of trimethylarsine oxide is completely different from arsenocholine and the tetramethylarsonium cation. Further increase to pH 7.7 does not change the k'-value (Fig. 6). Above pH 3 trimethylarsine oxide could undergo deprotonation to become the neutral hydrated oxide (Eqs. 1, 2) that is retained weakly.

3-Carboxy-4-hydroxybenzenesulfonic acid

The pH-dependent retention behavior of trimethylarsine oxide between pH 2 and 4 is compar-

able to arsenocholine and the tetramethylarsonium cation. The k'-values of trimethylarsine oxide are between 0.14 and 0.34 k'-units smaller than those for the tetramethylarsonium cation. The k'-values for trimethylarsine oxide fell markedly from ~3.4 at pH 2 to 1.6 at pH 3 and decreased further to 1.1 at pH 4. At pH 2 and 2.5 double peaks were observed.

3.6. Retention of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid with the mobile phases containing benzenesulfonates

Anionic arsenic compounds that may also be present in environmental samples may generate signals at short retention times that could overlap with signals from cationic arsenic compounds. Therefore, the retention behavior of arsenite, arsenate, methylarsonic acid, and dimethylarsinic acid with the mobile phases containing benzenesulfonates were studied.

Arsenite

With all investigated mobile phases, the k' for arsenite (0.57 ± 0.03) is independent of pH (Fig. 7). This behavior is expected, because arsenous acid with a pK_a of 9.2 is present as neutral H_3AsO_3 throughout the pH range 2–5, does not form an ion-pair, and is hardly retained.

Arsenate

The k' for arsenate decreased from approximately 0.5 at pH 2 to 0.2 at pH 5 with the investigated mobile phases (Fig. 7). The low k'-values and their decrease with increasing pH are caused by the negative charge on the analyte and the dissociation behavior of the arsenic acid (pK_a^1 2.2).

Methylarsonic acid

The k'-value for methylarsonic acid was constant between pH 2 and 4 and decreased from approximately 0.7 at pH 4 to approximately 0.3 at pH 5 with the investigated mobile phases. This behavior can be understood in terms of the pK_a of 4.1 for methylarsonic acid.

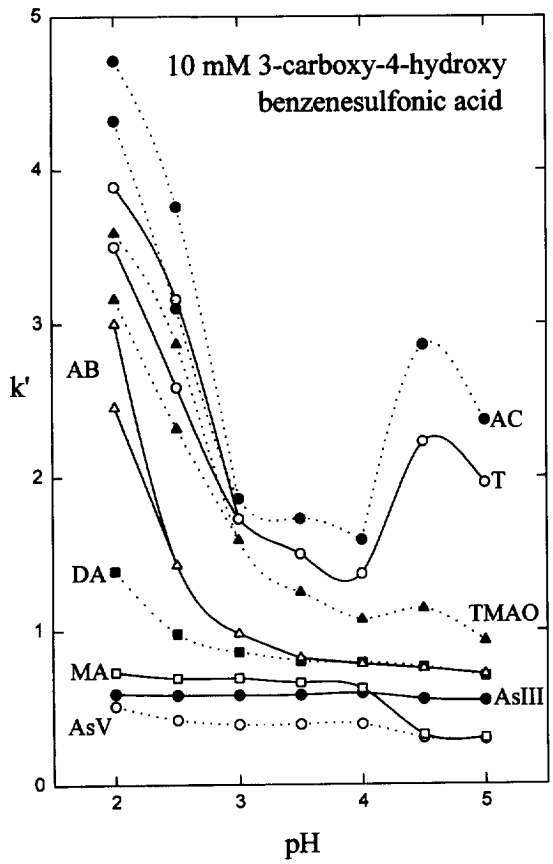

Fig. 7. Dependence of the k'-values of arsenite (As III), arsenate (As V), methylarsonic acid (MA), dimethylarsinic acid (DA), arsenobetaine (AB), arsenocholine (AC), trimethylarsine oxide (TMAO), and tetramethylarsonium (T) on the pH of 10 m*M* 3-carboxy-4-hydroxybenzenesulfonic acid (column, PRP-1, 250× 4.1 mm I.D.; flow-rate, 1.5 $cm^3\ min^{-1}$; detector, FAAS at 193.7 nm; loop, 100 μl; 5 μg As of each compound injected separately).

Dimethylarsinic acid

An increase of pH from 2 to 3 brought about a pronounced decrease in k' (between 0.16 and 0.53 k'-units depending on ion-pairing reagent) for dimethylarsinic acid. Between pH 3 and 5 the k'-values were almost constant at approximately 0.8 (Fig. 7). With a pK_a of 6.3 dimethylarsinic acid cannot be negatively charged in the pH range 2 to 5. The relatively high k' of 1.4 at pH 2 could be the result of a small apparent charge caused by protonation of a small fraction of dimethylarsinic acid. The

decrease of the k'-values between pH 2 and 3 would then be attributable to the conversion of $[(CH_3)_2As(OH)_2]^+$ to neutral $(CH_3)_2AsO_2H$.

3.7. Optimized conditions for the separation of arsenic compounds

The pH of the mobile phase and the ion-pairing reagent must be chosen to achieve – if possible – baseline separation of all arsenic compounds within a reasonable time. Because of the hydrophobic nature of ion-pairs with benzenesulfonate, the retention times are unacceptably long. With 4-hydroxybenzenesulfonate as ion-pairing reagent overlapping signals cannot be avoided. Arsenocholine, the tetramethylarsonium cation and trimethylarsine oxide produce broad signals. Arsenite and methylarsonic acid have the same retention time. The double peaks occurring for arsenocholine and the tetramethylarsonium ion with 3-carboxybenzenesulfonate as ion-pairing reagent make a clean separation impossible.

3-Carboxy-4-hydroxybenzenesulfonic acid mobile phases proved to be the best ion-pairing reagent. Chromatograms obtained with solutions of all eight arsenic compounds (5 μg As for each compound in 100 μl injected) showed, that with a mobile phase 10 mM with respect to the ion-pairing reagent at pH 3.5 arsenate, methylarsonic acid, arsenobetaine, trimethylarsine oxide, the tetramethylarsonium cation, and arsenocholine can be separated from each other (almost always to baseline) within 3 min (Fig. 8). When arsenite is also present, its signal overlaps with the signal from methylarsonic acid. The presence of arsenite is always indicated (unless the arsenite/methylarsonic acid ratio is low) by a shoulder. The signals from arsenobetaine and dimethylarsinic acid overlap completely. Under the same conditions but at pH 2.5 dimethylarsinic acid and arsenobetaine are base-line separated. The signals from arsenite and methylarsonic acid still overlap. The signals for trimethylarsine oxide, the tetramethylarsonium ion, and arsenocholine are too broad to be useful for quantification. Thus six arsenic compounds (seven if either arsenite or methylarsonic acid is absent) can be separated and quantified in two separate chromatographic experiments. If methylarsonic acid and arsenite are present, anion-exchange chromatography is recommended for the identification and quantification of these arsenic compounds [8].

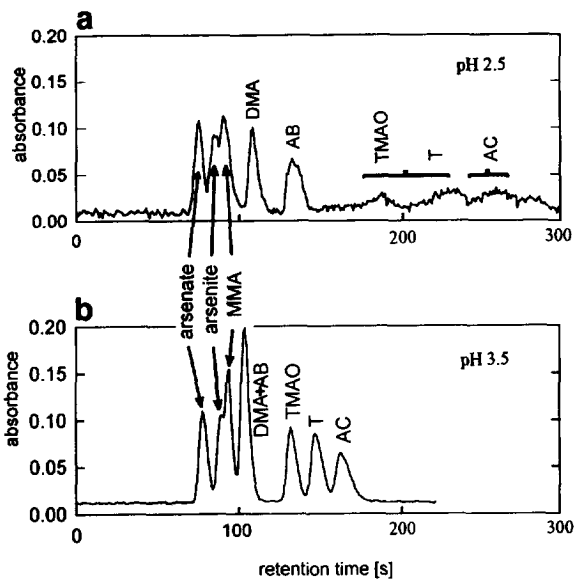

Fig. 8. Separation of arsenate, methylarsonic acid/arsenite, dimethylarsinic acid/arsenobetaine, trimethylarsine oxide, the tetramethylarsonium cation and arsenocholine (5 μg As each) with 10 mM aqueous 3-carboxy-4-hydroxybenzenesulfonic acid of pH 2.5 (a) and 3.5 (b) (column, PRP-1, 250×4.1 mm I.D.; flow-rate, 1.5 cm^3 min^{-1}; detector, FAAS at 193.7 nm; loop, 100 μl; 5 μg As of each compound injected).

The optimized chromatographic procedure with 3-carboxy-4-hydroxybenzenesulfonate as ion-pairing reagent was successfully used for the determination of arsenobetaine, the tetramethylarsonium ion, trimethylarsine oxide, and arsenocholine in 1:1 diluted seawater samples [25].

Acknowledgments

Dr. Gyula Vigh is gratefully acknowledged for helpful comments during the preparation of the manuscript.

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