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SPECIATION OF ARSENIC COMPOUNDS BY HPLC WITH HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY AND INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY DETECTION

XIAO-CHUN LE, WILLIAM R. CULLEN* and KENNETH J. REIMER

Department of Chemistry, 2036 Main Mall, University of British Columbia, Vancouver, B.C., Canada V6T 1Z1, and Environmental Sciences Group, Royal Roads Military College, FMO, Victoria, B.C., Canada V0S 1B0

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Summary—An arsenic specific detection system utilizing on-line microwave digestion and hydride generation atomic absorption spectrometry (MD/HGAAS) is described for arsenic speciation by using high performance liquid chromatography (HPLC). Both ion exchange chromatography and ion pair chromatography have been studied for the separation of arsenite, arsenate, monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA), and arsenobetaine (AB). When the commonly used mobile phases, phosphate and carbonate buffers at pH 7.5, are used on an anion exchange column, arsenite and AB co-elute. However, selective determination of these two arsenic compounds can be achieved by using the new detection system. Partial separation between arsenite and AB can be achieved by increasing the mobile phase pH to 10.3 and by using a polymer based anion exchange column. The detection limit obtained by using anion exchange chromatography with MD/HGAAS detection is approximately 10 ng/ml (or 200 pg for a 20-μl sample injection) for arsenite, DMAA and AB, 15 ng/ml (or 300 pg) for MMAA, and 20 ng/ml (or 400 pg) for arsenate. Complete separation of the five arsenic compounds is achieved on a reversed phase C18 column by using sodium heptanesulfonate as ion pair reagent. Comparable resolution between chromatographic peaks is obtained by using MD/HGAAS detection and inductively coupled plasma mass spectrometry (ICPMS) detection.

Chemical speciation of arsenic compounds continues to be an interesting and important topic of research,[1–3] mainly because the toxicity and availability of arsenic compounds is species-dependent. It has been demonstrated[2,4,5] that total arsenic concentration is not an appropriate measure for assessing toxicity, environmental impact, and effect of occupational exposure. However, due to the lack of techniques for efficient separation and sensitive detection of particular species, most environmental and clinical studies are based on the measurement of total arsenic concentration. The speciation of arsenic presents an analytical challenge particularly when environmental and biological systems are concerned, where often only trace amounts of arsenic compounds are present in complex matrices.

The coupling of high performance liquid chromatography (HPLC) with various spectrometries has proven very useful for chemical speciation studies because the combination takes advantage of both the separation power offered by HPLC and good selectivity and sensitivity obtainable by using modern spectrometry. Thus many HPLC detection systems employed for speciation studies make use of atomic spectrometry and mass spectrometry.[3,6–8] Atomic absorption spectrometry (AAS) with both flame and graphite furnace atomizers has been used as a method of HPLC detection for arsenic speciation studies.[6,9–12] The coupling between HPLC and AAS has been reviewed recently.[6,13–15]

Inductively coupled plasma atomic emission spectrometry (ICPAES) has been successfully coupled to HPLC for use in arsenic speciation.[16–19] The coupling is straightforward because the usual flow rate under which a HPLC operates, typically 1 ml/min, is compatible with the uptake flow rate of an ICPAES system. Although HPLC/ICPAES has been shown to be very useful, it does not have the high sensitivity that some trace analysis applications require:

*Author for correspondence.

for example, the speciation of arsenic in human urine requires working with samples where the total concentration of all arsenic species is often in the 10–20 ng/ml level.

The coupling of HPLC with ICPMS offers advantages because of the extremely high sensitivity, large dynamic range, and isotope ratio measurement capability that an ICPMS instrument can offer. Application of HPLC/ICPMS to arsenic speciation[20–29] has demonstrated the usefulness of this technique. However, wide acceptance of ICPMS as a HPLC detector in routine analysis probably requires substantial reduction of the current high cost of this instrument.

Hydride generation sample introduction is one of the most inexpensive and convenient methods that can be used to improve analytical sensitivity. By incorporating a hydride generation system as a post-column derivatization method into a HPLC system, sensitivity for arsenic speciation can be improved, whether the detection system is flame AAS,[30–35] electrothermal AAS,[36,37] or ICPAES.[38–42]

One major limitation in using hydride generation as a part of the HPLC detection system for arsenic speciation is that many environmentally and biologically important organoarsenic compounds such as arsenobetaine, arsenocholine, the tetramethylarsonium ion, and arsenosugars do not form volatile hydrides. Thus these arsenic species are not detected by using hydride generation. To solve this problem, an appropriate on-line decomposition procedure is required. In our previous study,[43] we developed a microwave decomposition method which rapidly and completely decomposes these organoarsenic compounds to arsenate, and arsenate can readily form a hydride, AsH_3. The on-line decomposition capability of the method was successfully tested by using flow injection analysis. In the present study, we further investigate the capability of the on-line microwave decomposition hydride generation AAS as a HPLC detector for arsenic speciation. For comparison, speciation by using HPLC/ICPMS is also described.

EXPERIMENTAL

Instrument

The HPLC system used consists of a Waters Model 510 solvent delivery pump, a Reodyne 6-port injector with a sample loop volume of 25 μl, a polymer-based anion exchange column (BDH PolySpher SAW, 12 cm × 4.6 mm i.d.) with a guard column packed with the same material for ion exchange chromatography and a C18 reversed phase column (Phenomenex 10 μ Bondclone C18, 30 cm × 3.9 mm i.d., 10 μm particle size) and a guard column of the same packing material for ion pair chromatography. The detectors for the HPLC were either a hydride generation atomic absorption spectrometer (HGAAS) or an inductively coupled plasma mass spectrometer (ICPMS).

A Varian AA-1275 atomic absorption spectrometer equipped with a deuterium background corrector and a Varian air-acetylene flame atomizer was used. An open-ended/T-shaped quartz tube was mounted above the AA burner and in the flame. Light from an arsenic hollow cathode lamp (Hamamatsu Photonics, Japan) was aligned to pass through the center of the quartz absorption tube (11.5 cm × 0.8 mm i.d.). Hydrides were introduced from the side arm of the T-shaped quartz tube. The hollow cathode lamp was operated at a 8-mA lamp current. Atomic absorption signals, measured at a wavelength of 193.7 nm were recorded on a Hewlett Packard 3390A integrator.

The HGAAS system for HPLC detection is schematically shown in Fig. 1. In Scheme (I) of the Fig. 1, effluent from the HPLC directly meets at two Teflon T-joints T1 and T2 (1/16″ joints, Mandel), with continuous flows of hydrochloric acid (A) and sodium tetrahydroborate(III) (B) introduced by using a peristaltic pump (P) (Gilson Minipuls 2). Upon mixing the HPLC effluent, acid and tetrahydroborate(III) solutions, hydride generation takes place. A

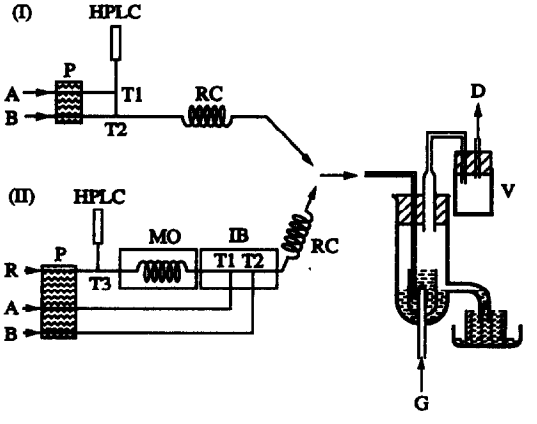

Fig. 1. Schematics of HPLC/HGAAS system (I) and HPLC/MD/HGAAS system (II) A—hydrochloric acid B—$NaBH_4$ R—digestion reagent P—peristaltic pump T1, T2, and T3—Teflon T-joints (1/16″) MO—microwave oven RC—reaction coil IB—ice water cooling bath G—carrier gas (nitrogen) V—glass vial (10 ml) D—to detector (AAS)

complete reaction is achieved by using a 1-m reaction coil (RC) (1 mm i.d. PTFE coil) and the combined gas/liquid separator apparatus as described previously.[43] Hydride generated from the reaction is carried by a continuous flow of nitrogen carrier gas (G) to the atomic absorption spectrometer (D). Because the reaction solution in the gas/liquid separator is hot, particularly when the microwave oven decomposition is used on-line as in Scheme (II) of Figure 1, and some foaming is produced due to the use of heptanesulfonic acid as HPLC eluent, some aerosols are carried into the hydride transport line. To prevent signal fluctuation due to the accumulation of aerosols in hydride transport line, a small glass vial (V) (inner volume, 10 ml) is used between the hydride generator and the AAS to trap the aerosols.

In Scheme (II) of Fig. 1, the HPLC effluent undergoes microwave decomposition before the hydride generation takes place. As demonstrated previously,[43] a solution containing 3% $K_2S_2O_8$ and 0.1*M* sodium hydroxide efficiently decomposed organoarsenicals to arsenate with the aid of microwave energy, and this reagent mixture was used in the present study. The HPLC effluent and the decomposition reagent (R) meet at a T-joint (T3) (1/16", Mandel, Canada). This solution mixture flows through a PTFE decomposition coil (5 m × 0.8 mm i.d.) located in a continuously operating microwave oven (MO) (500 W, 2450 MHz, Sharp Electronics, Japan), where decomposition of arsenic compounds takes place. The hot solution from the microwave oven then meets the continuous flows of acid (A) and tetrahydroborate (B). An ice water cooling bath (IB) is used following the microwave decomposition in order to reduce dispersion and to reduce the amount of aerosol produced. After the hydride generation reaction and gas/liquid separation, arsines are introduced to the flame-heated quartz tube for atomic absorption measurement. The time that an analyte spends in the microwave decomposition hydride generation system is approximately 30 sec This was determined by by-passing the HPLC column and measuring the time between the injection of an arsenate standard and the appearance of the signal maximum.

A VG PlasmaQuad 2 Turbo Plus inductively coupled plasma mass spectrometer (ICPMS) (VG Elemental, Fisons Instrument) equipped with a SX300 quadrupole mass analyzer, a standard ICP torch (Fassel configuration) and either a Meinhard concentric nebulizer or a de Galan V-groove nebulizer was used. A minichiller (Coolflow CFT-25, Neslab) was used to cool the spray chamber to approximately 4°. The sampling position and ion lens voltages were optimized with respect to a signal-to-noise ratio at m/z 75 by introducing a solution containing 30 ng/ml of arsenite in 1% nitric acid. The quadrapole mass analyzer was operated in the single ion monitoring mode. The instrumental operating conditions are listed in Table 1. A PTFE tubing (20 cm × 0.4 mm i.d.) with appropriate fittings was used to directly connect the outlet of the HPLC analytical column to the inlet of the ICP nebulizer. Signals at m/z 75 were monitored by using a multichannel analyzer and data were automatically transferred to and stored in the VG data system. Once a chromatographic run was complete, a chromatogram was then plotted on a Epson FX-850 printer.

Standards and reagents

Standard solutions of arsenite, arsenate, MMAA, DMMA, and arsenobetaine, were prepared in 0.01*M* nitric acid. Sodium tetrahydroborate(III) (Aldrich) solutions in 0.1*M* sodium hydroxide (BDH) were prepared and filtered prior to use. Microwave digestion reagents containing $K_2S_2O_8$ (BDH) and NaOH were prepared fresh daily. All HPLC eluents, carbonate (BDH) buffer, phosphate (BDH) buffer, and heptanesulfonate (Aldrich) were prepared in distilled water and filtered through a 0.45-μm membrane. The pH of these eluents was adjusted by using sodium hydroxide and nitric acid. All the reagents used were of analytical reagent grade or better.

Procedures

For both anion exchange and ion pair chromatography, the columns were equilibrated with the corresponding eluent flowing at 1 ml/min for at least two hours before any sample injection was made. Detailed experimental conditions are listed in Table 1. For the speciation of arsenic in human urine, the urine samples were centrifuged and filtered through a 0.45-μm membrane filter to remove any suspended particulates before they were subjected to HPLC analysis. A guard column was always used preceding an analytical HPLC column. Arsenic compounds in the urine samples were identified by matching the retention times of chromatographic peaks of the urine samples with those of

Table 1. Summary of experimental conditions

HGAAS	*No Digestion*	*With Microwave Digestion*
HCl Conc. (Flow Rate):	2*M* (3.4 *ml/min*)	3*M* (3.4 *ml/min*)
$NaBH_4$ Conc. (Flow rate):	0.65*M* (3.4 *ml/min*)	0.65*M* (3.4 *ml/min*)
Carrier Gas Flow Rate:	160 *ml/min*	160 *ml/min*
Digestion Reagents:	none	0.1*M* $K_2S_2O_8$ & 0.4*M* NaOH (4 *ml/min*)
Digestion Coil:	none	5 m × 0.5 *mm* i.d.
Microwave Oven:	none	500 *W*
ICPMS (VG PQ2 Turbo Plus)		
Forward r.f. power:	1.35 *kW*	
Reflected power:	< 10 *W*	
Outer gas (Cooling) flow rate:	13.8 *l./min*	
Intermediate gas (Auxillary) flow rate:	0.70 *l./min*	
Nebulizer gas flow rate:	0.96 *l./min*	
Spray chamber:	water cooled to approximately 4°	
Sampling cone orifice diameter:	1.0 *mm*	
Skimmer cone orifice diameter:	0.75 *mm*	
Single ion monitoring mode:	*m/z* 75	
HPLC	*Anion Exchange*	*Ion Pair*
Column:	BDH PolySphere SAW 12 cm × 4.6 *mm* i.d.	Phenomenex C18 30 *cm* × 3.9 *mm* i.d.
Mobile Phase:	50*mM* phosphate or carbonabe buffer pH 7.5, 9.0 or 10.3	10*mM* heptanesulfonate 0.1% methanol, pH 3.5
Flow Rate	1.0 *ml/min*	1.0 *ml/min*

spiked standards. After urine sample analysis was completed, the anion exchange column was flushed with 120 ml of distilled water to remove any possible salt build up due to continued injection of urine samples. It was then flushed with 60 ml of 4m*M* sodium salicylate (pH 7.8) before it was stored in this eluent for later use. Likewise, the reversed phase C18 column was washed with 120 ml of distilled water followed by 60 ml of methanol/water (60/40) mixture. If the urine samples are expected to contain high concentration of protein, the column may be cleaned by running a urea solution (5m*M*) for 1 hr followed by methanol/water (60/40) mixture.

RESULTS AND DISCUSSION

Anion exchange chromatography

Phosphate[10,21,26,33,41,42] and carbonate[27,29,30,36] buffers have been commonly used as mobile phases to separate anionic arsenic compounds on an anion exchange column. Therefore, we chose these systems to begin our study on arsenic speciation and to evaluate the capability of on-line microwave decomposition/hydride generation atomic absorption spectrometric system (MD/HGAAS) as a HPLC detector. Figure 2 shows chromatograms obtained by using an anion exchange column and 50m*M* phosphate (pH 7.5) buffer as mobile phase. Figure 2(a) was obtained by using the set up shown in Scheme (I) of Fig. 1, where the HPLC effluent does not undergo microwave decomposition. Although five arsenicals were present in the injected standard, only four of these are detected and appear in the chromatogram; arsenobetaine does not form a hydride without prior decomposition to a hydride forming species[43] and therefore is not detected by HGAAS. When the on-line microwave decomposition system is used immediately before the hydride generation step [Scheme (II) of Fig. 1], arsenobetaine is decomposed to arsenate, a hydride forming species, and all the five arsenic compounds are detected [Fig. 2(b)]. The response of the system to arsenobetaine alone is shown in Fig. 2(c).

At pH 7.5, H_3AsO_3 remains undissociated as its pK_{a1} value is 9.3;[44] and arsenobetaine is probably present as a neutral zwitterion. Therefore, neither H_3AsO_3 nor arsenobetaine is retained on the anion exchange column and both co-elute in the void time (volume) of the system. This experiment was repeated by using an ICPMS as the HPLC detector, and similar results to those shown in Fig. 2(b) were obtained. Thus arsenobetaine and arsenite cannot be separated from each other on the anion exchange column by using a pH 7. 5 buffer as mobile phase. This problem has also been encountered by other workers;[26,27,41] yet it is often ignored and arsenobetaine is excluded from

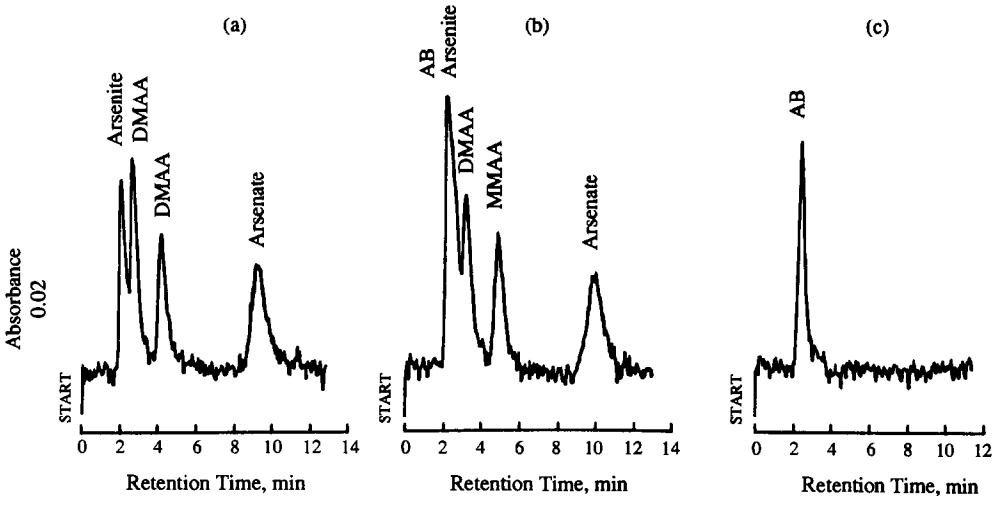

Fig. 2. Chromatograms of arsenic compounds obtained by using an anion exchange column with 50m*M* phosphate buffer (pH 7.5) as mobile phase and HGAAS detection. (a) 100 ng/ml of 5 arsenic species; no digestion; AB not detected, (b) 100 ng/ml of 5 arsenic species; with microwave digestion, (c) 150 ng/ml of AB; with microwave digestion.

many speciation studies even though it is environmentally and biologically a very important arsenic compound. It is certainly the major arsenic compound in many kinds of seafood.[2] The detection system developed here, however, allows an easy solution to the problem, and the concentration of each can be measured by difference, by making two chromatographic runs, one with on-line microwave decomposition of the HPLC effluent prior to HGAAS detection (Scheme II) and the other without microwave digestion (Scheme I). Thus, selective determination can be achieved by using the new system developed here, and a specific application of this is demonstrated below where the speciation of arsenic compounds in urine samples is described.

Figure 3 shows chromatograms obtained

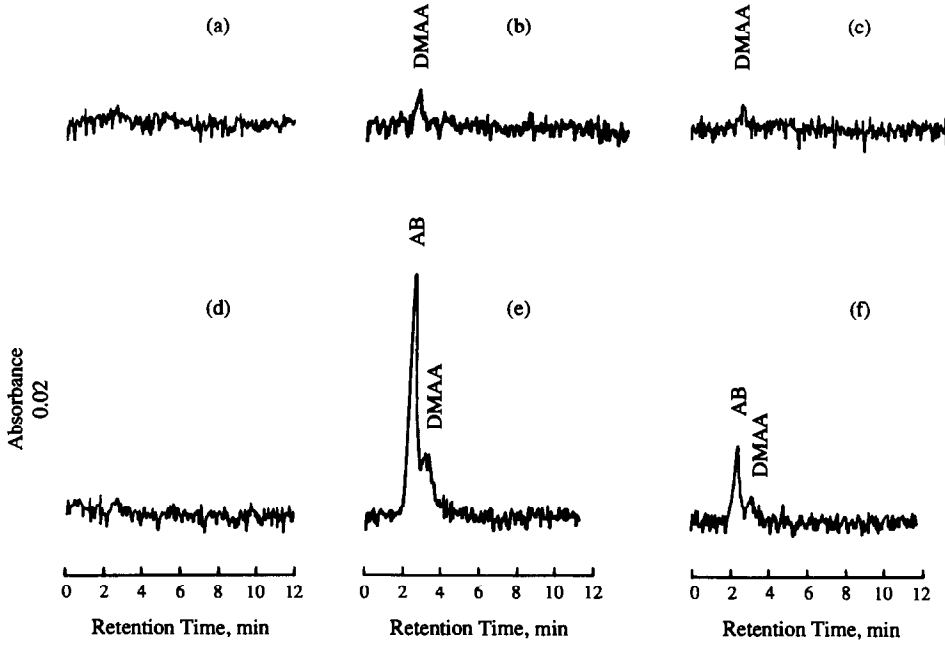

Fig. 3. Chromatograms obtained from urine samples collected 2 hr before the ingestion of crab meat (a) and (d), and 7 hr (b) and (e) and 17 hr (c) and (f) after the ingestion of crab meat. (a) (b) and (c)—no digestion, (d), (e), and (f)—with microwave digestion.

from urine samples collected 2 hr before (a) and (d), 7 hr (b) and e), and 17 hr (c) and (f) after a volunteer ingested some crab meat. The chromatograms (a), (b), and (c) were obtained without using microwave digestion; whereas (d), (e) and (f) were obtained after on-line microwave digestion. As is shown in Fig. 3, only a trace amount (less than 20 ng/ml) of DMMA is detected by using HGAAS without microwave decomposition. When the same samples were subjected to chromatography and MWD/HGASS determination, arsenobetaine is clearly detected in urine samples collected at 7 hr [Fig. 3(e)] and 17 hr [Fig. 3(f)] after the ingestion of crab meat. The first peaks in Fig. 3(e) and 3(f) account for approximately 120 and 37 ng/ml, respectively, of arsenic as arsenobetaine since arsenite is not detectable in the samples [Fig. 3(a)–(c)]. If there were detectable arsenite, the difference of the first peak in peak height (or peak area) between the second run (with microwave decomposition) and the first run (without microwave decomposition) would account for arsenobetaine. By using the detection system developed here for HPLC, all five arsenic compounds can be easily speciated with two chromatographic runs of approximately 12 min per run.

The chromatograms in Fig. 3 also illustrate the time course of the human urinary excretion of arsenobetaine after the ingestion of crab. Maximum arsenobetaine concentration in urine, 120 ng/ml is reached approximately 7 hr after the ingestion of crab meat [Fig. 3(e)]. Seventeen hours after the ingestion, the arsenobetaine in the urine is decreased to 37 ng/ml [Fig. 3(f)]. Analysis of urine samples collected beyond 30 hr after the ingestion of crab meat showed that the arsenobetaine concentration was reduced to below the detection limit of the method. Little variation is seen in the DMAA concentration, and there are no other arsenic compounds detected.

The detection limits, defined as three times the standard deviation of the blank, are approximately 10 ng/ml (or 200 pg for a 20-μl sample injection) for AB, DMAA, and arsenite, 15 ng/ml (or 300 pg) for MMAA and 20 ng/ml (or 400 pg) for arsenate by using the HPLC/MD/HGAAS system.

Effect of mobile phase pH

Separation of arsenic compounds by using anion exchange chromatography is usually performed with a mobile phase at around neutral

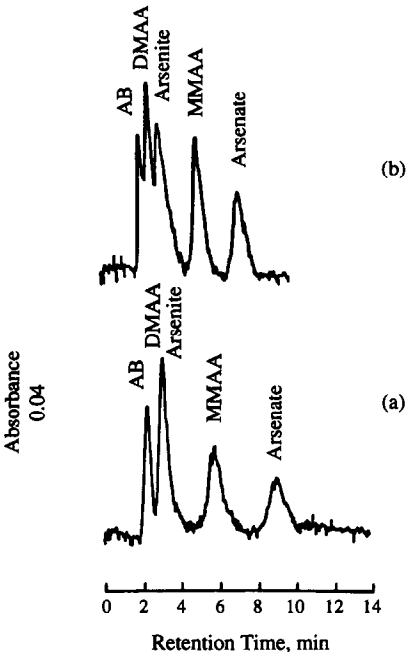

Fig. 4. Chromatograms of four arsenicals obtained by using a polymer based anion exchange column and 50m*M* phosphate buffer, at two pH's, as eluent. No microwave decomposition was used. (a) pH 9.0; 400 ng/ml of 4 arsenicals, (b) pH 10.3; 200 ng/ml of 4 arsenicals.

pH. Although it is known that the pH of the mobile phase is very important for the separation, very little work has been done to systematically study the effect of pH. This is partly because, in most of the studies, silica based anion exchange columns were used, limiting the mobile phase to a narrow pH range near neutral. However, with the use of a polymer based column, mobile phases in the pH range 1–14 can be used without column deterioration. Figure 4 shows chromatograms from four arsenicals when 50m*M* phosphate buffers at pH 9.0 and 10.3 are used as mobile phase. When the pH is increased from 7.5 [Fig. 2(a)] to 9.0 [Fig. 4(a)], arsenite and DMAA co-elute. This is consistent with the results of Sheppard *et al.*,[27] who concluded that pH 7.5 is preferred for the separation of these two species on a silica-based anion exchange column. However, our results show that when the mobile phase pH is further increased to 10.3 [Fig. 4(b)] chromatographic peaks from DMAA and arsenite are separated again, and a longer retention is observed for arsenite. The increased retention of arsenite has an advantage in that this species is now separated from arsenobetaine. This is shown in Fig. 5 where five arsenic species are determined by using HPLC/MD/HGAAS. Arsenite and

DMAA co-elute at pH 9.0 [Fig. 5(a)], whereas arsenite and arsenobetaine co-elute at pH 7.5 [Fig. 2(b)]. When the pH is increased to 10.3 [Fig. 5(b)] a stronger retention of arsenite results in arsenobetaine, arsenite and DMAA being partially separated from one another and being well separated from MMAA and arsenate.

The pK_{a1} value of arsenious acid (H_3AsO_3) is reported[44] to be 9.3. At pH 7.5, very little H_3AsO_3 is dissociated so the species is not retained on the column and is eluted in the void volume. Arsenobetaine, a zwitterion, is also co-eluted in the void volume. As the pH is increased, more H_3AsO_3 is dissociated to give the anion $H_2AsO_3^-$, which is retained more strongly on the anion exchange column than the undissociated species. Arsenobetaine remains a zwitterion within the pH range studied and is eluted in the void column. Thus arsenite and arsenobetaine are separated from each other.

Further increase of pH to 10.9 resulted in the partial overlap of peaks from arsenite and MMAA. In the pH region below 7.5, no improvement was observed in the separation of the five arsenic compounds. Therefore, for optimum separation, the pH of the mobile phase should be 10.3.

Similar results were obtained when 50m*M* sodium bicarbonate buffer was used as mobile phase: no difference was found in the chromatographic separation when using either phosphate or carbonate buffers.

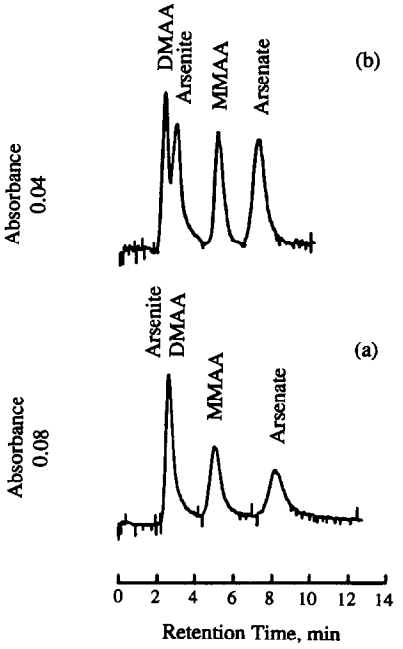

Fig. 5. Chromatograms of five arsenicals (200 ng/ml each) obtained by using a polymer based anion exchange column and 50m*M* phosphate buffer, at two pH's, as eluent. On-line microwave decomposition was used. (a) pH 9.0, and (b) pH 10.3.

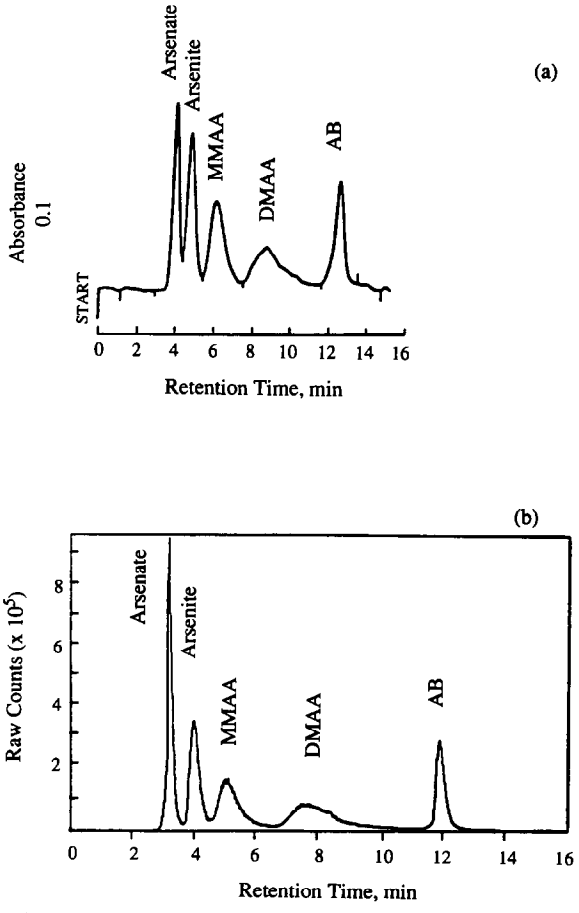

Fig. 6. Chromatograms of five arsenicals (1 μg/ml each) obtained on a reversed phase C18 column with 10m*M* heptanesulfonate (pH 3.5), an ion pair reagent, as eluent. (a) with MD/HGAAS detection, (b) with ICPMS detection.

Ion pair chromatography

The anion exchange chromatography system described above is simple, but it is convenient only for studies involving arsenite, arsenate, MMAA and DMAA. Although the new detection system developed can be used to speciate arsenobetaine as well, it requires two chromatographic runs. Consequently we have studied ion pair chromatography in order to improve the separation of arsenic compounds. An anion pair chromatographic system that uses heptanesulfonate as an ion pairing reagent was studied and the separation conditions were optimized. Figure 6 shows chromatograms of five arsenic compounds that are well separated on a reversed phase C18 column and are detected by

using MWD/HGAAS [Fig. 6(a)] and ICPMS [Fig. 6(b)]. The similar resolution in the two chromatograms indicates that the dispersion in the detection systems is comparable. This is probably because the average time that an analyte spends in the microwave digestion hydride generation unit is only 30 sec.

CONCLUSION

The combination of HPLC with MD/HGAAS system has been demonstrated to be very useful for arsenic speciation. The system is easy to construct and inexpensive. While the use of ICPMS as a detection system for HPLC has been well-recognized, the cost of an existing commercial ICPMS instrument (both initial purchase and operating) makes it one of the most expensive HPLC detectors, and it may not be realistic for commercial application to dedicate an ICPMS as HPLC detector. The HPLC/MD/HGAAS system provides an attractive alternative for routine arsenic speciation, since this system is also capable of selectively determining some arsenic compounds, which is particularly advantageous when these compounds co-elute or are not completely separated by HPLC.

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