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Short communication

Ion chromatographic separation and determination of phosphate and arsenate in water and hair

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Abstract

A simple and sensitive method for the sequential determination of phosphate and arsenate was developed based on initial ion chromatographic separation followed by detection as the ion-association complex formed by heteropolymolybdophosphate and arsenate with bismuth. With 200 μl sample injection and separation on a AS4A-SC column using an eluent of 3.5 m*M* sodium hydrogen carbonate–10.0 m*M* sodium hydroxide, the detection limits which are calculated as the concentration equivalent to twice the baseline noise, were found to be 0.8 μg/l and 4.2 μg/l for P and As, respectively. *Spiked samples were analyzed and recoveries were found to be satisfactory in the range of 95–105% for phosphate and 90–105% for arsenate.* Samples of water *and hair* were analyzed by the proposed method.

Keywords: Phosphate; Arsenate

1. Introduction

Arsenic is toxic to living systems. If ingested, it tends to accumulate in certain parts of the body. Biological samples of blood, tissues, urine, nail, hair are considered as indicators of arsenic poisoning [1]. The LAFA in a study conducted in seven countries has brought out the significance of hair mineral analysis (in which As is included), as a means for assessing internal body burdens of environmental pollutants [2]. Based on a study of 2059 hair samples, Bozsai has reported that a good correlation exists between As in hair and that in water supply [3]. Because of its toxic nature, WHO has put a limit of 0.05 pg/l arsenic in drinking water. On the other hand, phosphate is an important nutrient for biological growth and is present in all natural waters. Many of the chemical reactions of phosphorous and arsenic, when present as phosphate and arsenate, are common. For example, phosphate and arsenate react with molybdate to yield heteropolymolybdate, which on reduction can form ion association complexes with bismuth or antimony. Thus, the separation of phosphate and arsenate is very often required. It is in this context that ion chromatographic separation in combination with suitable detection methods appears attractive. Not only that, ion chromatography is suitable for speciation studies, which are relevant from the point of view of bioavailability and toxicity.

Scanning the literature, ion chromatographic separation for arsenic followed by its determination in

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natural water and biological samples by AAS, ICP-AES, ICP-MS etc. has been reported [4–10]. The methods, though attractive, require high cost instrumentation. In a few cases, ion chromatographic separation followed by conductivity detection has been used [11,12]. Li et al., while reviewing the methods on speciation of arsenic, proposed an ion chromatographic separation followed by sequential determination of As(III) electrochemically and As(V) based on ion association complex formed by heteropolymolybdoarsenic acid with bismuth [13]. Regarding phosphate, there are a few papers related to ion chromatographic separation with conductivity detection [14–16]. However, no attempt has been reported for the sequential determination of As and P when present together. Therefore in the present work, an ion chromatographic separation of phosphate and arsenate followed by a simple spectrophotometric determination of the reduced species of the ion-association complex formed with ammonium molybdate and bismuth is presented. During this, the method of Li et al. is also improved. The present method has been applied for the determination of phosphate and arsenate in natural water and human hair samples.

2. Experimental

2.1. Instrumentation

The layout of the ion chromatograph Dionex model DX-30, coupled to a post column reactor and UV–VIS detector is shown Fig. 1. The separating column was AS4A-SC in combination with a SC guard column. The reagent flow through the post column reactor is kept constant by maintaining 60 p.s.i. of nitrogen gas.

The experiments are run at an eluent flow-rate of 1.65 ml/min using a degassed solution of 3.5 m*M* sodium hydrogen carbonate and 10.0 m*M* sodium hydroxide. The wavelength used for the spectrophotometric measurement is 700 nm. The output data is recorded through an integrator connected to the spectrophotometer. Optimization of all the parameters for better colour development is carried out.

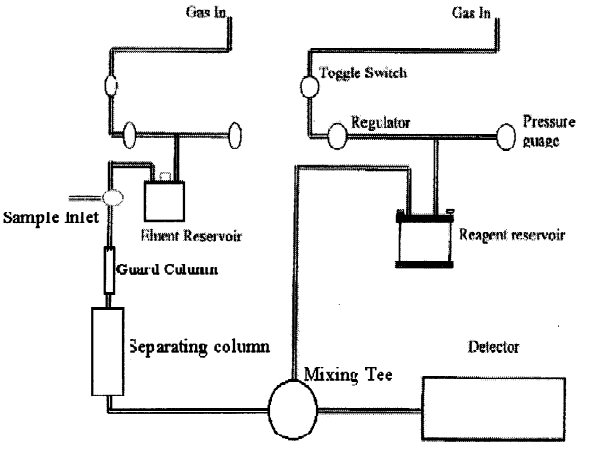

Fig. 1. Layout of the ion chromatograph.

2.2. Standard solutions and reagents

A standard phosphorus solution of 1000 mg/l is prepared by dissolving 0.439 g of predried potassium hydrogen phosphate in water and making up to 100 ml.

A standard As(V) solution is prepared by dissolving 0.42 g of sodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) in water and standardised by titration with standard iodine. This was suitably diluted to give a solution of 1000 mg/l.

2.2.1. Colour developing reagent

A 1.25 g measure of ammonium molybdate is dissolved in 48 ml of 9.0 *M* H_2SO_4. To this solution, 1.9 g of ascorbic acid was added followed by the addition of 12.5 ml of 10% $Bi(NO_3)_3$. Finally the solution was diluted to 250 ml. The solution is degassed just before use. It can be stored for 24 h.

2.3. Procedure

2.3.1. Water samples

Inject 200 μl of sample solution containing P (100–1000 μg/l) and As(V) (500–2000 μg/l) into AS4A column and elute with 3.5 m*M* $NaHCO_3$–10.0 m*M* NaOH at the flow-rate of 1.65 ml/min. Using a post column reactor, mix the effluent of the column with colour forming reagent and record the chromatogram with the spectrophotometer set at 700 nm.

The retention times for P and As are 5.37 min and 9.18 min, respectively.

2.3.2. Hair samples

Collect human hair sample and free it of extraneous contamination by washing with ether–acetone followed by EDTA solution as optimised by Caroli from different washing procedures [17]. Weigh 0.25 g of the sample and digest with 3 ml HNO_3 for 4 h and evaporate to dryness. Dissolve the residue in 3.5 m*M* $NaHCO_3$–10.0 m*M* NaOH (eluent) and make up to 25 ml. Inject 200 μl of the sample solution into the ASA4 column and follow the same procedure given for water samples.

3. Results and discussion

3.1. Effect of eluent concentration and flow-rate

Preliminary studies with various concentration ratios of $NaHCO_3$ and Na_2CO_3 indicated that the resolution between P and As(V) is optimum at a concentration of 3.5 m*M* $NaHCO_3$ and 10.0 m*M* NaOH.

The effect of flow-rate on the peak heights of P and As in the chromatogram is investigated by varying the flow-rate from 1.3 to 1.8 ml/min. The peak height is optimum at 1.65 ml/min and all further experiments were carried out using this flow-rate.

3.2. Post column reagent

A suitable colour developing system for the spectrophotometric determination of As(V) based on the ion association complex formed by heteropolymolybdoarsenic with bismuth in the presence of Triton X-100 is reported [13]. Preliminary studies for this indicated that Triton X-100 can be avoided without compromising the sensitivity. Hence it was decided to optimize the concentrations of other reagents.

3.2.1. Acid concentration

H_2SO_4 medium gave better absorbance than HNO_3 medium. Therefore the H_2SO_4 concentration was varied from 0.5 *M* to 3.0 *M* while injecting 500 μg/l of P and 1000 μg/l of As(V) into IC and detecting at 700 nm using a spectrophotometer. As shown in Fig. 2, the absorbance remains constant in the range of 1.5 to 2.5 *M* for P and 1.5 to 2.0 *M* for As. Hence, it was decided to use 1.75 *M* H_2SO_4.

3.2.2. Ammonium molybdate concentration

The ammonium molybdate concentration was varied from 0.25 to 1.5% (w/v) in the colour developing reagent while determining 500 μg/l of P and 1000 μg/l of As(V). The peak height increases with increase in ammonium molybdate concentration from 0.1 to 0.5% (w/v) and remains fairly constant on subsequent increase. Hence, 0.5% (w/v) ammonium molybdate solution was used in subsequent investigations.

3.2.3. $Bi(NO_3)_3$ concentration

It was found from preliminary studies that bismuth gives a better absorbance signal than antimony. The influence of bismuth nitrate on the determination of P and As was investigated by varying the bismuth nitrate from 0.1 to 1.0% (w/v) in the colour developing reagent. Fig. 3 shows the increase in absorbance signal with the increase in Bi from 0.1 to 0.5% (w/v). It remains fairly constant with further increase. As excess amount of bismuth nitrate is found to cause drift in absorbance, probably, because of

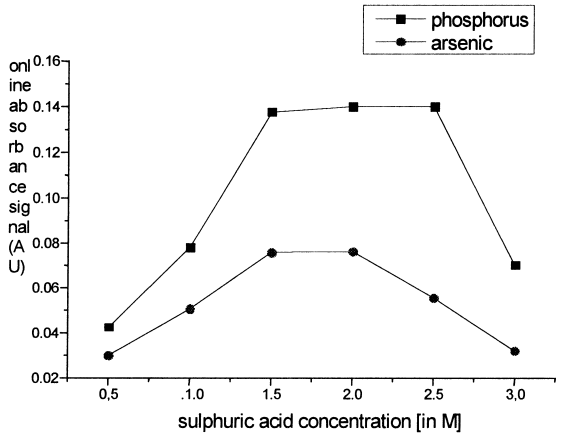

Fig. 2. Change in absorbance with respect to sulphuric acid concentration.

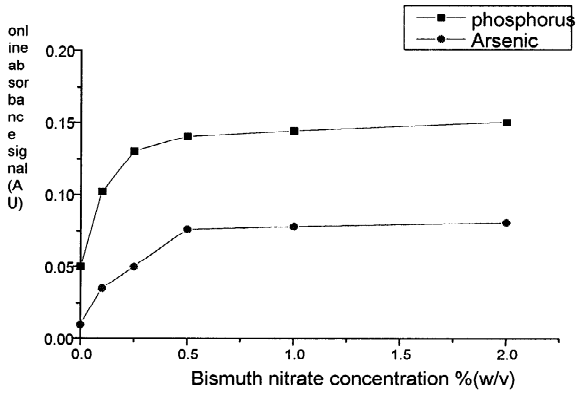

Fig. 3. Change in absorbance with respect to bismuth nitrate concentration.

hydrolysis. Hence 0.5% (w/v) bismuth nitrate was chosen for all further experiments.

3.2.4. Ascorbic acid concentration

Ascorbic acid had no influence on the absorbance of the colouring system when its concentration was varied from 0.5 to 1.0% (w/v). Therefore, 0.75% of ascorbic acid solution was used in subsequent experiments.

3.3. Calibration graph, precision, recovery and detection limit

Linear calibration graphs were obtained over the ranges of 100–1000 μg/l of P and 500–2000 μg/l of As(V). The relationship between peak height and concentration was obtained by the least square method, the scale being adjusted to 100 mV for 0.1 absorbance. In the case of phosphate, $y = (1.2784)x + 61.2$ with a correlation coefficient of 0.999 and for arsenate, $y = (0.3763)x + (-13.025)$ with a correlation coefficient of 0.998. Fig. 4 shows typical chromatogram on the separation and detection of 500 μg/l of P and 1000 μg/l of As(V) under our experimental conditions.

A standard solution containing 500 μg/l of P and 1000 μg/l of As(V) was analyzed six times successively under the same experimental conditions. *Precision in terms of* the relative standard deviation (RSD) was found to be 3.6% for P and 3.9% for As(V) respectively.

Tables 1 and 2 show the recoveries of phosphate and arsenate from spiked samples. The recoveries are 95–105% for phosphate and 90–105% for arsenate.

Under our experimental conditions, the detection limit which is defined as the concentration that gives

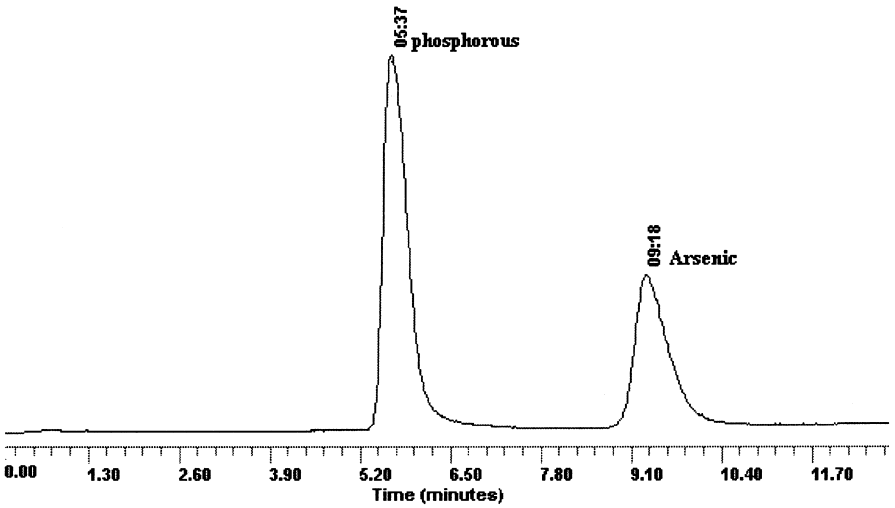

Fig. 4. Chromatogram of As(V) and P. Eluent 3.5 m*M* $NaHCO_3$–10.0 m*M* NaOH. Flow-rate 1.65 ml/min, sample loop 200 μl. As(V) 1000 μg/l; P 500 μg/l.

Table 1
Analysis of water samples

	Phosphate added (μg/l)	Phosphate found (μg/l)	*Recovery (%)*	Arsenate added (μg/l)	Arsenate found (μg/l)	*Recovery (%)*
Tap water	–	8.8		–	N.D.	
	10	19.2	*102*	25	25.8	*103*
	20	30.4	*105*	50	51.2	*102*
Well water	–	16.8		–	N.D.	
	12	27.4	*95*	25	26.4	*105*
	24	40.8	*100*	50	51.0	*102*

the peak intensity twice the baseline noise is 0.8 μg/l for P and 4.2 μg/l for As(V) respectively. *This may be compared with those reported by earlier workers. The detection limits are 0.17 μg/l of As(III) and 0.31 μg/l of As(V) in ion exchange chromatography (IC) followed by hydride generation AAS [4]. IC with ICP-MS gave detection limits of 0.04 to 0.06 μg/l of As [5]. IC with conductivity and spectrophotometric detection gave detection limits of 2.9 μg/l for As(III) and 13.0 μg/l for As(V) [13]. In the case of phosphate, a detection limit of 1 μg/l is reported using ion-exchange separation followed by conductivity detection [16].*

3.4. Interference studies

A systematic study of the interferences on the ion-exchange separation and determination of 500 μg/l of P and 1000 μg/l of As(V) was carried out. The interferences tested included the common anions—chloride, nitrate, sulphate and silicate. Except sulphate, none of the others interfere even if present at 5000-fold concentrations. More than 1000-fold amounts of sulphate suppress particularly the arsenic signal. High sulphate content samples may be injected after a suitable dilution depending on the concentrations of P and As.

3.5. Analysis of water samples

The developed procedure was used to analyse tap water and well water samples for P and As. The water samples were filtered through 0.2 μm Millipore filter immediately after collection. A standard addition study was also conducted to see the recoveries. Table 1 shows the actual and spiked values for P and As in real samples. Satisfactory recoveries of 92–102% for P and 90–95% for As were obtained.

3.6. Analysis of hair sample

The developed procedure was further applied to determination of P and As in human hair. Table 2 shows the actual and spiked values of P and As for real samples.

4. Conclusion

A simple IC procedure has been developed for the determination of phosphate and arsenate. This is the first time that an online sequential ion chromatographic separation with spectrophotometric determination of phosphate and arsenate is reported. The

Table 2
Analysis of hair samples

S.No.	Phosphate added (μg/g)	Phosphate found (μg/g)	*Recovery (%)*	Arsenate added (μg/g)	Arsenate found (μg/g)	*Recovery (%)*
1	–	22.5		–	0.1	
1	1.0	23.5	*100*	2.0	1.8	*90*
1		39.5		–	0.3	

procedure is based on the ion chromatographic separation of phosphate and arsenate on an anionic column AS4A using 3.5 m*M* $NaHCO_3$–10.0 m*M* NaOH as the eluant and spectrophotometric determination of the reduced species of the ion association complex of heteropolymolybdophosphorus (arsenic) acid with bismuth. Detection limits are found to be 0.8 μg/l for P and 4.2 μg/l for As. The method can be successfully applied to natural water and hair samples.

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