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A SIMPLE MANIFOLD FLOW INJECTION ANALYSIS FOR DETERMINING PHOSPHORUS IN PRESENCE OF ARSENATE

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A simple manifold flow injection analysis (FIA) for determining phosphorus in presence of arsenate in water, by the on-line reduction of As(V) to As(III) using sodium sulphite. The FIA method is applicable for the determination of phosphorus in water samples containing less than 0.37 $\mu g \cdot mL^{-1}$ *of* AsO_4^{3-}. *A solution obtained by mixing 6.30 g of* Na_2SO_3 *(1 M), 5.0 ml* H_2SO_4 *18 M completed up to 50 ml with deionized distilled water was used to reduce on line As(V) to As(III), using a reactor of 0.30 m. The limit of detection is* $0.05584 \pm 0.00167 \mu g \cdot mL^{-1}$ $P - PO_4^{3-}$ *and sampling frequency is 45 samples per hour. It is a simple and low cost methodology, easily applicable in the determination of phosphorus in samples of water contaminated with arsenate.*

Keywords: arsenate, contamination, flow injection, phosphorus, water.

1. Introduction

Phosphorus determination is a subject of great interest in the environmental field. This element is an essential nutrient for the growth of plants and animals, playing a major role in the process of eutrophication [1 – 3]. Phosphorus species determination by batch methods involves tedious and time-consuming steps. By contrast, flow techniques provide precise, accurate and rapid phosphorus determination with a minimal sample handling. Thus, several methods for determining nutrients such as nitrogen [4], [5] and phosphorus [6 – 11] in water and other matrixes based on flow injection analysis (FIA) have been reported.

On the other hand, spectrophotometry is the most common detection technique used in flow analysis since the apparatus required is simple, robust, and can be adapted easily for in situ determination. Phosphate determination by spectrophotometric methods usually involves the reaction between phosphate ions and molybdate in acidic conditions to form a heteropolyacid. The flow-injection analysis by the molybdenum blue method (MB) with ascorbic acid is included among the standard methods for water and waste water analyses [12]. This method has been used frequently as a conventional detection reaction of microamounts of phosphorus. For example, such a method was used for the simultaneous determination of phosphate and arsenate [13], [14], phosphate and silicate [15], [16].

Arsenate forms similar compounds with molybdate and phosphate and, therefore, can interfere positively in phosphate determinations by either method [17]. This interference may be of concern in areas affected by arsenic contamination though can be eliminated by reducing As(V) to As(III) before being measured. In the ascorbic acid method, treating arsenate with sodium thiosulphate has been shown to reduce quantitatively concentrations under 225 g $\cdot$ L^{-1} to As (III) within 15 min [18]. However, in acidic solutions thiosulphate undergoes a decomposition reaction that produces colloidal sulphur [19]. Other strategies to reduce As(V) to As (III) have been proposed; for example, sodium sulphite in the determination of phosphate by the solvent extraction/spectrophotometric with malachite green method (MG) [20], or the sodium dithionite solution in the MB method [21]. In the latter, only does complete arsenate reduction occur when the solution has been heated at 80°C for 20 min, and cooled to room temperature before being introduced in the spectrophotometric detector. In Argentina, there are large areas with their groundwater contaminated with arsenic, whose origin is thought to be the presence of volcanic ash from the Quaternary Age [22]. As groundwater is the main source of drinking water, the presence of arsenic interferes in phosphorus determination by the MB method.

Though there exist different alternatives to reduce As(V) to As(III) in determining phosphorus by the MB method, sodium sulphite has been applied only in the MG method. On the other hand, it is always possible to improve and simplify the configuration of a FIA system and make use of some alternative reagent in order to optimize and enhance its performance. Thus, this paper proposes a simple manifold FIA for determining phosphorus in the presence of arsenate in drinking water by reducing on-line As(V) to As(III) using sodium sulphite.

2. Experimental

2.1. Apparatus. A schematic diagram of the flow injection system is shown in Fig. 1. The manifolds were equipped with 3 Gibson Minipuls peristaltic pumps, one 5020 Rheodyne six-way injection valve, and 0.8 mm id Teflon tubes with their respective connectors and pump tubes. The spectrophometric readings were obtained using an USB 4000 Ocean Optic detector equipped with a 1 mm light path flow cell. Then, a mechanical stirrer and an USB 4000 Ocean Optic detector equipped with a 1 cm light path cell were used for validation.

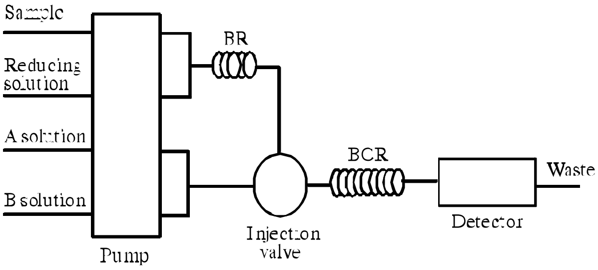

Fig. 1. Flow injection system for phosphorus determination.

2.2. Reagents and solutions. All the chemicals used in this study were reagents of analytical degree supplied by "Merck" (Darmstadt,Germany) while the solutions were prepared using distilled water. The stock calibration solution concentration was 100 $\mu g \cdot mL^{-1}$ $P - KH_2PO_4$ which was added 5 drops of toluene to reduce microbial activity. Out of these working solutions were prepared by adding distilled water as needed. The so-called A solution was prepared by mixing 1.8 g of ammonium molybdate $[(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O]$, 5.7 ml of 18 M H_2SO_4 and distilled water up to complete 100 ml; B solution was prepared by mixing 0.0277 g of antimony potassium tartrate $[K(SbO) \cdot C_4H_8O_6 \times 1/2\ H_2O]$ (0.010 g of antimony), 1.06 g de ascorbic acid ($C_6H_8O_6$) and distilled water as needed to complete 100 ml. Additionally, a solution out of 6.30 g of sodium sulfite (Na_2SO_3), 5.0 ml of sulfuric acid (H_2SO_4) and distilled water as needed to complete 50 ml was used to reduce As(V) to As(III). To optimize the system, solutions of 0.50 $\mu g \cdot mL^{-1}$ of $P - PO_4^{3-}$ and 0.20 $\mu g \cdot mL^{-1}$ of $As - AsO_4^{3-}$ as well as mixtures of both (0.50 and 0.20 $\mu g \cdot mL^{-1}$) were used, while to study the method interference and applicability solutions of $As - AsO_4^{3-}$ (KH_2AsO_4) at different concentrations were used.

2.3. Sample preparation. Sampling was made in both urban and rural areas of the Robles Department, Santiago del Estero city, Argentina in accordance with the prevailing norms (ISO 5667-1). Containers previously washed without detergent and rinsed with the groundwater flowing through them for one minute were used to collect the sample of groundwater. The samples were kept in refrigerator until analysis.

2.4. Procedures. The flow injection system used for determining phosphorus is shown in Fig. 1. In this manifold a modification to the FIA system for determining phosphorus previously proposed by Lopez Pasquali, 2004; was introduced tending to make the on-line reduction of As(V) to As(III) possible. Both the A and B reactive solutions enter together and act as a carrier solution. The sample is combined with a reducing agent in the coil (BR) before being injected. It reacts subsequently with the color reagent (carrier solution) in the reactor (BCR). The stream passes through the flow cell where the colored compound is measured at 880 nm. Both the chemical and physical variables affecting the analytical performance of the proposed system were carefully studied and optimized. To optimize the univariate analysis was used. All the measures were made in triplicate.

To study the maximum tolerable concentration of As – AsO_4^{3-} in the method proposed in this paper, As – AsO_4^{3-} solutions ranging between 0.20 and 0.40 $\mu g \cdot mL^{-1}$ (corresponding to an AsO_4^{3-} concentration varying between 0.37 and 0.74 $\mu g \cdot mL^{-1}$) were prepared and added to P – PO_4^{3-} solutions of 0.50 $\mu g \cdot mL^{-1}$ free of foreign substances. In turn, the standard Murphy and Riley manual method was used [12], [18] to validate the FIA method and the F test was applied to determine significance.

3. Results and discussion

3.1. Optimization of parameters. To optimize the system, parameters already determined in previous papers [9] were fixed as follows: flow rates: 1.60 $mL \cdot min^{-1}$ (sample) and 0.32 $mL \cdot min^{-1}$ (A and B solutions); injection volume: 200 ml; BCR length: 0.55 m; A and B solutions chemical composition. As to optimizing working conditions, the system's behavior was analyzed and changes in the content of both H_2SO_4 and Na_2SO_3 making up the reducing solution were introduced as well as the BR length when injecting.

3.1.1. Variation of H_2SO_4 volume in the reductive solution. The length for the BR was fixed at 0.25 m and the volume of H_2SO_4(C) added to the solution of Na_2SO_3 ranged between 4.0 and 7.0 ml. Fig. 2 shows the absorbance obtained when the solutions of P – PO_4^{3-}, As – AsO_4^{3-} and their mixture were injected versus the volume of H_2SO_4 added.

It is seen that absorbance decreases as the volume of acid increases though this behavior is less steeper between 5.0 and 6.0 ml. The results obtained after injecting the solutions of P – PO_4^{3-} and the mixture are similar especially between 5.0 and 5.5 ml H_2SO_4 whereas those obtained for the solution of As –

AsO_4^{3-} are substantially lesser and become negative after adding 5.0 ml of H_2SO_4.

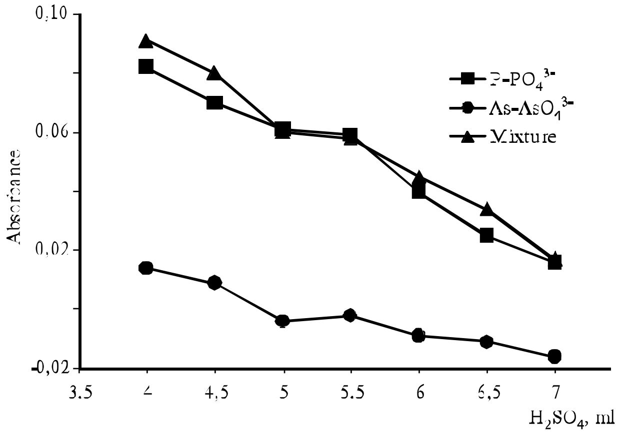

Fig. 2. Absorbance vs H_2SO_4 added volume.

The results show a similar behavior with the P – PO_4^{3-} and mixture solutions with a hardly perceptible level of interference by arsenate by the addition of 5.0 ml of H_2SO_4. This is why this volume of sulphuric acid is considered the most appropriate volume to prepare those 50 ml of reducing solution.

3.1.2. Variation of Na_2SO_3 in the reductive solution. Once the volume of H_2SO_4 was fixed at 5.0 ml, the amount of Na_2SO_3 used to prepare 50 ml of reducing solution was modified (Fig. 3). It's observed that when the amount of sodium sulphite increases so it does the absorbance of the solutions of P – PO_4^{3-} and mixture while absorbance starts decreasing at 5.23 g of Na_2SO_3 for the solutions of As – AsO_4^{3-} and mixture. Again the curves for the P – PO_4^{3-} and mixture solutions are similar and both reach their peaks when 6.30 g Na_2SO_3 (1.0 M) is used. On the other hand, the figures obtained for arsenate are noticeably lesser.

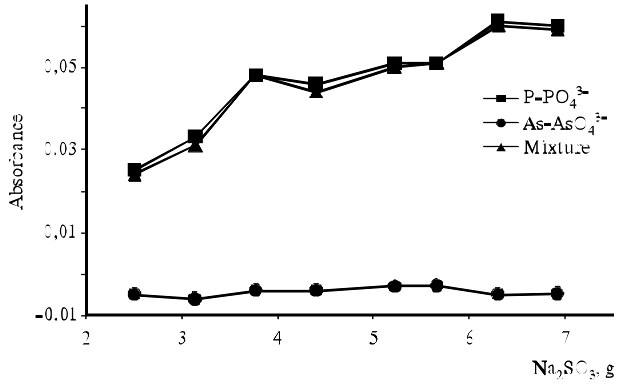

Fig. 3. Absorbance solutions P – PO_4^{3-}, As – AsO_4^{3-} and mixture vs Na_2SO_3 quantity (g).

According to these results, it might be established that when using 6.30 g of Na_2SO_3 the same maximal signal is obtained for both $P - PO_4^{3-}$ and mixture solutions which would show that in using this concentration the arsenate is reduced to arsenite without interfering in phosphorus determination. From this, it can be said that the solution prepared out of 6.30 g of Na_2SO_3, 5.0 ml H_2SO_4 and distilled water to complete 50 ml is the optimal to make As(V) reduce to As(III) in the system proposed in this paper.

3.1.3. Optimization of BR length. Once the reducing solution concentration was defined, the system behavior was studied by varying the BR length between 0.20 and 0.40 m. Fig. 4 depicts the absorbance obtained in terms of the BR reducing coil length. It is seen that as the BR length increases up to 0.35 m both $P - PO_4^{3-}$ and mixture solutions decrease intensities; at such point their values start increasing. In the case of arsenate, the signals obtained are substantially smaller than those corresponding to the other two solutions including negative values and a minimal response at 0.30 m.

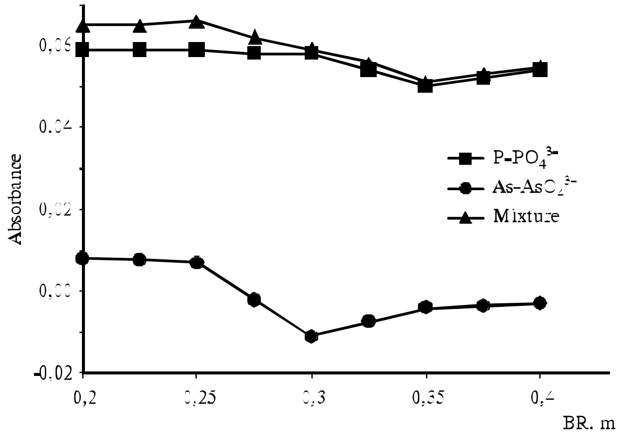

Fig. 4. Absorbance vs BR length.

These results would indicate that arsenate does not interfere in phosphorus determination when a 0.3 m long BR is used; therefore it is this which is considered the most appropriate value.

3.2. Maximum arsenate tolerance concentration in phosphate detection. To define the limiting concentration over which the arsenate starts interfering phosphate determination using the FIA system proposed in this paper, a 0.50 $\mu g \cdot mL^{-1}$ $P - PO_4^{3-}$ solution (blank) and 0.20; 0.30 and 0.40 $\mu g \cdot mL^{-1}$ $As - AsO_4^{3-}$ solutions were scanned along the 450 and 950 nm wavelength range.

The results show that at $As - AsO_4^{3-}$ concentrations higher than 0.2 $\mu g \cdot mL^{-1}$ (equivalent to 0.37 $\mu g \cdot mL^{-1}$ of AsO_4^{3-}) the interference brought about by this analyte might not be eliminated by proposed method. Taking into account that the maximum tolerable arsenic limit in water for human consumption stablished by World Health Organization (WHO) is 0.01 $\mu g \cdot mL^{-1}$, it follows that only in highly contaminated water samples and this method would not be applicable.

3.3. Analytical Perfomance. The analytical performance of the system was evaluated in terms of linear range, the detection limit, and reproducibility. The linearity intervals were defined between 0.10 and 2.0 $\mu g \cdot mL^{-1}$ $P - PO_4^{3-}$ and the detection limit was estimated [23] and gave LOD = (0.05584 ± 0.00167) $\mu g \cdot mL^{-1}$ $P - PO_4^{3-}$. The reproducibility and precision values were 2.63 and 2.56% respectively. The sampling frequency was 45 samples per hour.

3.4. Determination of $P - PO_4^{3-}$ *in water samples and validation.* The method developed in this paper was applied to water samples from Santiago del Estero, Argentina, showing contamination with arsenic. The average concentrations of phosphorus as phosphate obtained by the standard (CST), and FIA (CFIA) methods, and the difference between both (AM) are shown in Table. It's observed that the results obtained by applying both methods are similar with errors smaller than 5% at a 95% of confidence.

$P - PO_4^{3-}$ concentration in groundwater obtained by standard and FIA methods and Δ_M

Samples	$P - PO_4^{3-}$ ($\mu g \cdot mL^{-1}$)		Δ_M
	Standart Method	FIA Method	
1	0.405 0.003	0.391 0.003	0.014
2	0.38 8 0.008	0.432 0.003	-0.044
3	0.352 0.011	0.400 0.001	-0.048
4	0.41 0 0.002	0.429 0.004	-0.019
5	0.37 8 0.007	0.331 0.003	0.047
6	0.409 0.003	0.398 O.002	0.011
7	0.390 0.005	0.381 0.004	0.009
8	0.411 0.003	0.405 0.005	0.006
9	0.403 0.005	0.389 0.004	0.014
10	0.38 5 0.003	0.378 0.005	0.007

The validation results were: $F_c = 2.33$ and $F_t = 4.03$ (F_c and F_t stand for the experimental and theoretical values respectively). As $F_c < F_t$ it follows that there is no significant difference between both methods.

4. Conclusions

The manifold FIA proposed in this paper is appropriate to determine P – PO_4^{3-} in samples of water with AsO_4^{3-} concentrations lower than 0.37 μg · mL^{-1}, that might be applicable to water samples with higher arsenic content.

It turned out to be a precise and reproducible method with a detection limit of 0.05584 ± 0.00167 μg · mL^{-1} of P – PO_4^{3-} within a linearity range of 0.10 and 2.0 μg · mL^{-1} of P – PO_4^{3-}. The simplicity of the system proposed, together with the on line reduction of As(V) to As(III) with sodium sulphite, speeds up the analysis and reduces its cost.

Additionally, it proved to be a simple and low cost methodology, easy to apply for determining phosphorus in samples of arsenic contaminated water for consumption.

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