Phosphite Determination in Fertilizers after Online Sequential Sample Preparation in a Flow Injection System

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A flow injection spectrophotometric system is proposed for phosphite determination in fertilizers by the molybdenum blue method after the processing of each sample two times on-line without and with an oxidizing step. The flow system was designed to add sulfuric acid or permanganate solutions alternately into the system by simply displacing the injector−commutator from one resting position to another, allowing the determination of phosphate and total phosphate, respectively. The concentration of phosphite is obtained then by difference between the two measurents. The influence of flow rates, sample volume, and dimension of flow line connecting the injector−commutator to the main analytical channel was evaluated. The proposed method was applied to phosphite determination in commercial liquid fertilizers. Results obtained with the proposed FIA system were not statistically different from those obtained by titrimetry at the 95% confidence level. In addition, recoveries within 94 and 100% of spiked fertilizers were found. The relative standard deviation ($n = 12$) related to the phosphite-converted-phosphate peak alone was $\leq 3.5\%$ for 800 mg L^{-1} P (phoshite) solution. Precision due to the differences of total phosphate and phosphate was 1.1% for 10 mg L^{-1} P (phosphate) + 3000 mg L^{-1} P (phosphite) solution. The sampling rate was calculated as 15 determinations per hour, and the reagent consumption was about 6.3 mg of $KMnO_4$, 200 mg of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 40 mg of ascorbic acid per measurement.

KEYWORDS: Fertilizer; phosphite; FIA; spectrophotometry

INTRODUCTION

Phosphorus is essential to plant nutrition and is considered to be a critical element because natural phosphate-based fertilizers usually offer low bioavailability of P for plants to absorb (*1, 2*). Traditionally, the source of phosphorus in most crops has been phosphate-based fertilizers (*3*). Nevertheless, phosphorous acid and its salts have been used alternatively to phosphates because phosphites are absorbed by the leaves and roots much more quickly than phosphates, may act as fungicides, and play a role in the maturation of fruits, disease resistance, yield, and other quality aspects of agroindustrial products (*4*). In this context, the availability of accurate analytical methods of analysis for phosphite may improve laboratory skills and enhance their capability to obtain high-quality analytical data. Therefore, the production and commercialization of phosphite-based fertilizer in the agribusiness field has gained force.

The analytical techniques usually employed for phosphite determination are gravimetry (*5*), titrimetry (*6*), amperometry (*7, 8*), and spectrophotometry (*9, 10*). The gravimetric methods avalilable in the literature are based on either $NH_4MgPO_4 \cdot 6H_2O$ or $Hg_2Cl_2(s)$ formation. The former method requires a previous oxidation of phosphite to phosphate using wet ash digestion procedures. The mercurous chloride method can be used without sample pretreatment, but it generates large amounts of Hg-based residue, counteracting the tendency toward a clean chemistry (*11*). The application of iodimetry to determine phosphite in fertilizers is found (*6*). This method is feasible provided the concentrations of Mn^{2+} and humic substances are lower than 150 and 20 mg L^{-1}, respectively. A palladium film on a Pt electrode has been proposed for manual determination of phosphite by amperometry (*8*), but the low adherence of the Pd film at the surface of the Pt electrode impaired the application of this electrode in routine analysis of workable samples.

Regarding spectrophotometry, the phosphomolybdenum blue (PMB) or molybdovanadophosphoric acid (MVP) methods can be used for phosphite provided phosphite is converted to phosphate prior to the determination (*9*). Online oxidation of phosphite by potassium permanganate solution was the basis of a spectrophotometric procedure for phosphite determination by flow injection analysis (FIA) (*10*). This system cannot be straightforwardly applied to samples containing phosphate at fairly high concentrations because it is impossible to distinguish phosphite from phosphate. In this system, the manual change of permanganate by an acidic solution allows phosphite determination, but it is time-consuming and increases analytical costs.

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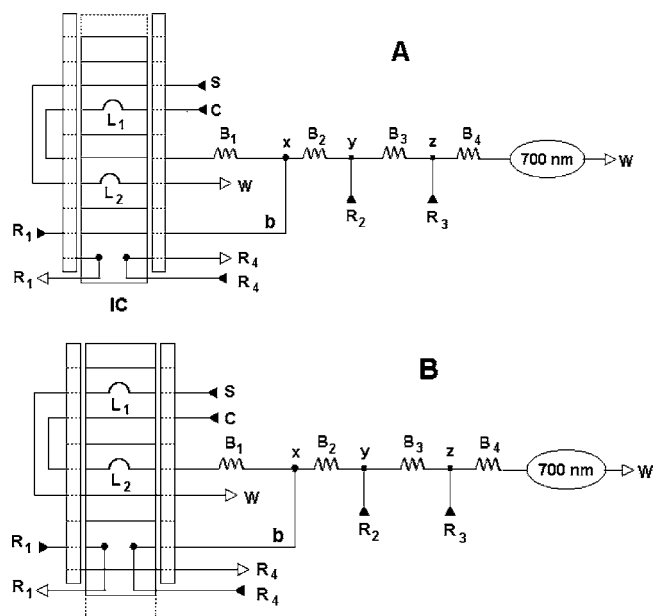
Figure 1. Flow diagram of the system for phosphite determination: IC, injector–commutator; L_1, L_2, sampling loops (100 μL); S, sample or analytical solutions (3.0 mL min^{-1}); R_1, 1.0 mol L^{-1} H_2SO_4 solution (1.0 mL min^{-1}); R_2, 5.0% (w/v) $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ + 1.0 mol L^{-1} H_2SO_4 solution (1.0 mL min^{-1}); R_3, 1.0% w/v of ascorbic acid (1.0 mL min^{-1}); R_4, 1.0×10^{-2} mol L^{-1} $KMnO_4$ + 1.0 mol L^{-1} H_2SO_4 solution (1.0 mL min^{-1}); C, water (1.0 mL min^{-1}); W, wastes; x, y, z, confluent points; B_1, B_2, B_3, and B_4, reaction coils (650 mm $\times$ 0.8 mm i.d.; 500 mm $\times$ 0.8 mm i.d.; 300 mm $\times$ 0.8 mm i.d.; 300 $\times$ 0.8 mm).

These shortcomings can be circumvented by using a FIA system involving sequential measurement of phosphite and phosphate. Reports of sequential detection of phosphite and phosphate by spectrophotometry are lacking in the literature.

The feasibility of this strategy is demonstrated in the present paper, which reports the development of a flow method for phosphite determination in agroindustrial samples. Two equal aliquots of sample are sequentially processed in a FIA system in the presence and absence of $KMnO_4$ solution, and the difference between heights of the resulting transients gives the concentration of phosphite. The performance of the proposed procedure was checked after commercial liquid fertilizers were analyzed.

EXPERIMENTAL PROCEDURES

Reagents, Analytical Solutions, and Samples. High-purity water obtained using a Millipore Rios 5 reverse osmosis and a Millipore Milli-Q Academic system (resistivity = 18.2 MΩ cm), high-purity chemicals, and sulfuric acid of analytical grade (Mallinckrodt) were used throughout to prepare solutions.

A 1.0 mol L^{-1} sulfuric acid solution (R_1, **Figure 1**) was prepared by appropriate dilution of the concentrated acid.

A 5% (w/v) ammonium molybdate solution (R_2, **Figure 1**) was daily prepared by dissolving 5.0 g of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Merck, Darmstadt, Germany) in 80 mL of 1.0 mol L^{-1} sulfuric acid and making the volume up to 100 mL with the same acid solution.

A 1% (w/v) solution of ascorbic acid (R_3, **Figure 1**) was daily prepared by dissolving 1.0 g of $C_6H_8O_6$ (Mallinckrodt, Paris, KY) in 100 mL of water.

A $1.0 \ 10^{-2}$ mol L^{-1} $KMnO_4$ solution (R_4, **Figure 1**) was prepared weekly by dissolving 158.04 mg of $KMnO_4$ (Merck) in about 80 mL of 1 mol L^{-1} sulfuric acid and making up the volume up to 100 mL with the same acid solution. This solution was kept in an amber bottom and filtered before use.

The sample carrier stream (C, **Figure 1**) was water.

A 4000 mg L^{-1} P (phosphite) stock standard solution was prepared by dissolving 27.871 g of $Na_2HPO_3 \cdot 5H_2O$ (Riedel-deHaën) in about 400 mL of water, and the volume was completed to 1000 mL with water.

A 1000 mg L^{-1} P (phosphate) stock standard solution was prepared by dissolving 4.582 g of Na_2HPO_4 (Merck) in about 400 mL of water, and the volume was completed to 1000 mL with water.

Five working standard solutions containing phosphite at concentrations of 0.0, 500, 1000, 2000, and 4000 mg L^{-1} P were weekly prepared in water by appropriate dilution of the stock standard solutions.

Six commercial liquid fertilizer samples were supplied by Samaritá Industry and Trading Ltd. (Artur Nogueira, Brazil). Two grams of sample was dissolved in about 200 mL of water, and thereafter the volume was completed to 1000 mL with water.

Recovery tests for spiked fertilizer samples were also investigated after the addition of 1−5 mL of a 4000 mg L^{-1} P (phosphite) standard solution to 100 mL of diluted samples (500 times).

FIA System. The flow system depicted in **Figure 1** comprised an Ismatec (Zurich, Switzerland) IPC-8 multichannel peristaltic pump equipped with Tygon or Viton (this for R_1) pumping tubes, a manual injector−commutator (*12*), a Femto (São Paulo, Brazil) 482 spectrophotometer with a U-shaped flow cell (10 mm optical path; ≈100 μL of lighted volume), a Kipp and Zonen (Delft, The Netherlands) BD 111 strip chart recorder, poly(tetrafluoroethylene) (PTFE) tubing (i.d. = 0.8 mm), reaction coils, and accessories. In the position specified in **Figure 1**, 100 μL of sample (S), selected by sampling loop L_1, is injected into carrier stream C (**Figure 1A**), and the established sample zone merges sequentially with reagents R_1, R_2, and R_3 at confluent points x, y, and z, respectively. Phosphate species present in the sample react under acidic conditions with ammonium heptamolybdate and ascorbic acid, forming the PMB complex inside the reactor B_4. Passage of the colored complex (*13*) through the flow cell of spectrophotometer (700 nm) results in a transient peak proportional to only phosphate present in the sample. After peak maximum measurement, the injector−commutator IC is switched to the other position (**Figure 1B**) and a second sample volume (L_2, 100 μL) is injected in the presence of an intermittent flow of permanganate solution (R_4, **Figure 1B**). Phosphite is oxidized to phosphate inside the coiled reactor B_2 and, then, phosphate reacts sequentially with R_2 and R_3, forming a PMB complex inside the reactor B_4. In this situation, the recorded peak corresponds to phosphate and phosphite. The concentration of phosphite is calculated by difference.

After system dimensioning, the proposed procedure was applied to phosphite determination in liquid fertilizers. In addition, recovery tests for phosphite were also investigated. All transient measurements were in peak height and triplicate.

FIA Conditions. With the system shown in **Figure 1**, the influence of the main parameters related to the FIA system performance, such as length of the reaction coil B_1, reaction time, flow rates, and sample volume, was investigated. Concentration and order of addition of reagents were not investigated in this work. They were selected on the basis of previous studies (*10*).

The potential of the proposed FIA system for the sequential determination phosphite and phosphate was studied by processing in the system pure working standard solutions of phosphate (10 mg L^{-1}) and phosphite (3000 mg L^{-1}) and a working standard solution containing both anions at the same concentration.

After the parameters had been selected, the main analytical characteristics were evaluated. The repeatability was evaluated after 12 successive injections of a diluted sample containing ca. 800 mg L^{-1} P (phosphite). Accuracy was checked by applying the proposed procedure to the direct determination of phosphite in liquid fertilizer samples, which were also analyzed by titrimetry (*6*). In addition, phosphite recovery tests were also carried out on fertilizer samples. The limits of detection (LOD) and quantification (LOQ) were calculated according to the IUPAC recommendation (*14*). All measurements of transient absorbance were made at least in triplicates. The consumption of reagents and sample, dispersion, and sampling rate were calculated according to theoretical aspects of FIA (*15*).
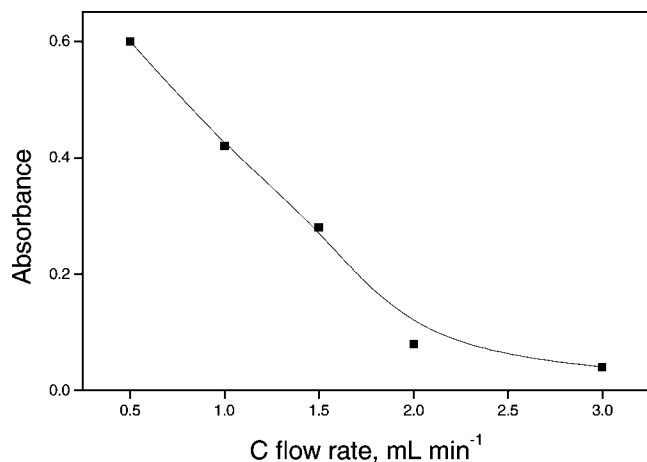
Figure 2. Influence of the flow rate of carrier C on absorbance. Absorbance values correspond to a 2000 mg L^{-1} P (phosphite) working solution processed with the FIA system of **Figure 1**.

RESULTS AND DISCUSSION

To simulate the manual determination of phosphorus by the PMB method, the order of addition of reagents adopted in the proposed procedure was as follows: potassium permanganate, ammonium molybdate, and ascorbic acid. The concentrations of R_2, R_3, and R_4 were fixed at 5.0% (w/v) $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.0% (w/v) ascorbic acid, and 1.0×10^{-2} mol L^{-1} $KMnO_4 + 1.0$ mol L^{-1} H_2SO_4, respectively, as suggested in previous work involving phosphate detection (*10*).

Shown in **Figure 2** is the effect of variation of the flow rate of carrier C on absorbance (phosphite oxidation). Different flow rates of C were obtained by varying the rotation speed of the peristaltic pump in order to obtain different flow rates (0.5– 3.0 mL min^{-1}) for channel C. An extra peristaltic pump was used to maintain the flow rates of R_1, R_2, R_3, and R_4 fixed at 1.0 mL min^{-1}. The faster the rotation speed of the peristaltic pump is, the shorter the oxidation time. The height of analytical signals underwent reduction of about 80% when the C flow rate was varied from 0.5 to 2.0 mL min^{-1}, above which variations were negligible (**Figure 2**). As a compromise among sensitivity, reagent consumption, and sampling rate, the flow rate of carrier C selected for subsequent experiments was 1.0 mL min^{-1}. For the 100 μL sampling loop and 1000 mg L^{-1} of P (phosphite) standard, the transient signal measured at the maximum of the peak height was close to 0.25 absorbance. It should be noted that low flow rates may favor the formation of air bubbles along the analytical tubing and flow cell, impairing the detection. Therefore, in such situations, it is a good practice to use a small syringe at upright position after reaction coil B_4 to aid in air bubble removal. The cylindrical configuration of the syringe may improve the mixing conditions for the spectrophotometric detection.

The FIA system was designed to determine phosphite on the basis of a dual-run approach by adding sulfuric acid and potassium permanganate solutions intermittently. In this system, the sample is processed in the absence and presence of reagent R_4, generating two transient peaks that correspond to only phosphate and phosphite plus phosphate, respectively. Segment b of the FIA system depicted in **Figure 1** was used to connect the injector IC to the analytical path and was as short as possible (5 cm). Because reagents R_1 and R_4 were added alternately, reagent R_1 (sulfuric acid) could be contaminated with R_4 reagent (permanganate) inside the main analytical path, which could produce unwanted oxidation of phosphite to phosphate during
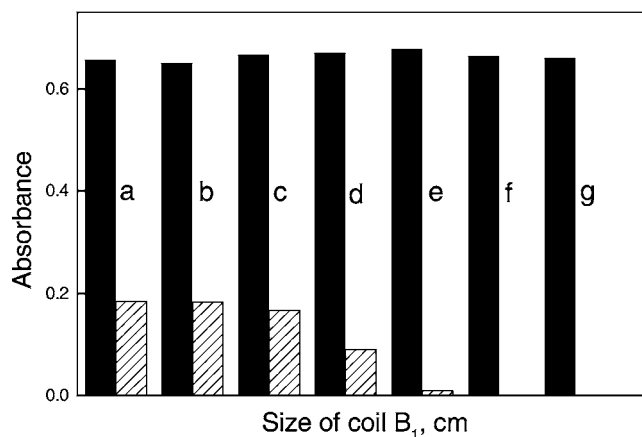
Figure 3. Influence of the size of reaction coil B_1 on absorbance. Bar heights correspond to absorbance ($n = 3$) of a 3000 mg L^{-1} P (phoshite) working solution measured in the presence of 1.0 10^{-2} mol L^{-1} $KMnO_4$ + 1.0 mol L^{-1} H_2SO_4 (black bar) and 1.0 mol L^{-1} H_2SO_4 (dashed bar) using 2.5 (a), 5 (b), 10 (c) 25 (d), 50 (e), 65 (f), and 100 cm (g) length for B_1.
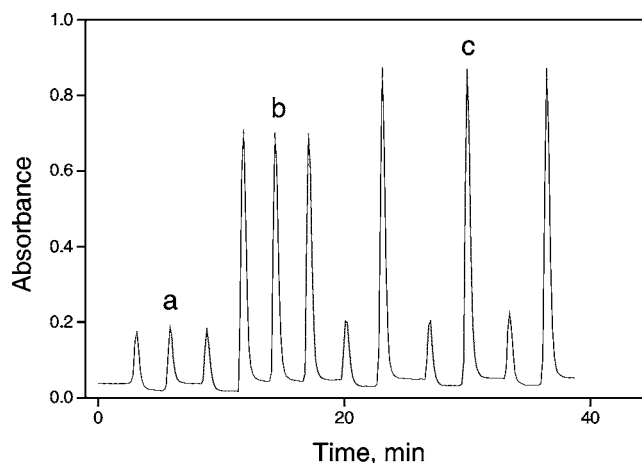
Figure 4. Sequential measurement of phosphite and phosphate. Transient signals refer to (a) 10 mg L^{-1} P (phosphate), (b) 3000 mg L^{-1} P (phosphite), and (c) 10 mg L^{-1} P (phosphate) + 3000 mg L^{-1} P (phosphite) working solutions injected in the FIA system of **Figure 1** in triplicate.

the only phosphate determination (**Figure 1A**). Therefore, the influence of the length of reaction coil B_1 on absorbance was investigated by varying the length of B_1 from 2.5 to 100 cm. There was a significant decrease (dashed bars) in the unwanted oxidation of phosphite with the increase of coil B_1 (**Figure 3**). The optimum size for B_1 was reached at 65 cm, the length chosen for further experiments.

The influence of injected sample volume on absorbance was investigated within the 50–200 μL range. A linear relationship between absorbance and phosphite concentration was observed up to 100 μL. Better sensitivity of the analytical curve was observed for 100 μL. This volume was selected for subsequent experiments. In this situation, the dispersion coefficient was calculated as 3.4 (*15*).

Shown in **Figure 4** are transient peaks associated with sequential measurement of phosphite and phosphate. From left to right, each set of transient signals corresponds to the following solutions injected in triplicate: (a) 10 mg L^{-1} P (phosphate), (b) 3000 mg L^{-1} P (phosphite), and (c) 10 mg L^{-1} P (phosphate) + 3000 mg L^{-1} P (phosphite). Analysis of the figure reveals that the peak height related to pure phosphate solution (set a) is similar to the lower peaks of set c obtained in the absence of

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Table 1. Comparative Results: Results (Mean ± Confidence Interval) for Phosphite in Percent (w/w) P_2O_5 in Liquid Fertilizers As Determined ($n = 3$) by the Proposed FIA System and by Titrimetry (*6*)

sample	value provided by the manufacturer[a]	FIA	titrimetry
1	20	19.4 ± 1.3	19.2 ± 1.6
2	30	29.5 ± 2.1	30.8 ± 1.3
3	35	35.4 ± 2.7	35.8 ± 2.7
4	40	40.4 ± 3.2	39.1 ± 2.4
5	20	19.5 ± 1.5	19.1 ± 1.2
6	28	28.2 ± 2.4	28.3 ± 1.8

[a] In % (w/w) P_2O_5 due to phosphite.

$KMnO_4$. For set c, the difference between signals obtained in the presence (highest peaks) and absence of permanganate (lowest peaks) corresponded to phosphite. In fact, this difference is similar to peak heights of pure phosphite solution (set b). These studies strengthen the initial proposition for phosphite determination by means of sequential online sample preparation in the FIA system.

With regard to selectivity, a 2000 mg L^{-1} P (phosphite) standard working solution plus 1000 mg L^{-1} Mn^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, or Cu^{2+} (concentrations at which they are known to be present in diluted fertilizer samples), no interference were observed. No interference effects were observed after the addition of 1000 mg L^{-1} carbonate, nitrate, chloride, and sulfate. Besides arsenic(V) and silicon interfere in the determination of phosphorus by the PMB method, this was not observed, probably due to the low levels of these anions present in the fertilizer samples and the large dilution (500 times) involved in this work. The typical calibration equation corresponding to analytical solutions in the 500–4000 mg L^{-1} P (phosphite) concentration range was $A = 0.007 + 2.068 \times 10^{-4}$[analyte], and the linear correlation coefficient was 0.9993. Accuracy was assessed by analyzing commercial liquid fertilizers (**Table 1**). The results obtained with the proposed procedure were not statistically different from those obtained by titrimetry at the 95% confidence level (*t* test). It is important to point out that values provided by the manufacturer are expressed in P_2O_5 due to phosphite. In addition, recoveries within 94 and 100% of spiked fertilizer were found. The phosphate contents in the samples were typically ≤0.5% (w/w) P_2O_5. These findings mean that no more than 2.5% of total phosphorus P_2O_5 content in samples analyzed is present as phosphate. The limits of detection of phosphate and phosphite are 0.13 and 9 mg L^{-1}, respectively, and the corresponding limits of quantification are 0.43 and 30 mg L^{-1}. The relative standard deviation ($n = 12$) related to phosphite-converted-phosphate peak alone was ≤3.5% for 800 mg L^{-1} P (phosite) solution. Precision due to the differences of total phosphate and phosphate was 1.1% for 10 mg L^{-1} P (phosphate) + 3000 mg L^{-1} P (phosphite) solution. The sampling rate of the FIA system was 15 h^{-1}, and reagent consumption was about 6.3 mg of $KMnO_4$, 200 mg of $(NH_4)_6$-$Mo_7O_{24}\cdot 4H_2O$, and 40 mg of ascorbic acid per measurement.

The proposed FIA system with alternate introduction of permanganate and sulfuric acid was effective in the determination of phosphite after online fertilizer processing two times. This strategy was easily put into practice simply by switching the conventional injector–commutator. The method can be considered an alternative to the use of conventional methods already available for phosphite determination. Although manual methods for phosphite are simple, they are time-consuming due to the laborious sample preparation. The proposed procedure in this work comprises an automated spectrophotometric system with online sample treatment for phosphite and phosphate determination. Besides the direct analysis, selectivity, low reagent consumption, and rapidity, high accuracy and precision were obtained due to repetitive operation of all steps involved. Among other benefits of automation, the possibility to identify the source of phosphorus in phosphite-based fertilizers is relevant to check their quality.

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