

## **Expedient flow-based amperometric method for reliable determination of orthophosphate in soil and water**

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### **Abstract**

In-line coupling of a flow injection manifold with amperometric detection is herein proposed for reliable and expeditious determination of orthophosphate in environmentally relevant samples. The analytical method is based on the in-line formation of 12-molybdophosphate which is subsequently reduced electrochemically at a glassy carbon electrode. Standard/sample solutions were injected into a stream of 0.1 mol/L potassium chloride and then merged downstream with 0.5 % (w/v) molybdate in 2.5% (v/v) sulfuric acid solution to form the measurant, that is, 12-molybdophosphate complex, in a reaction coil. Electrochemical reduction of the complex at a glassy carbon electrode at 0.20 V vs Ag/AgCl produced electrical current which was directly proportional to the concentration of orthophosphate in the injected solution which was continuously recorded as a transient signal. Linear calibration graphs spanned the ranges of 10-100  $\mu\text{g L}^{-1}$  P, 0.1-1.0  $\text{mg L}^{-1}$  P, and 1-10  $\text{mg L}^{-1}$  P on the basis of selected experimental conditions with a limit of detection of 3  $\mu\text{g L}^{-1}$  P. Relative standard

deviations for 11 replicate injections of both  $0.5 \text{ mg L}^{-1} \text{ P}$  and  $5 \text{ mg L}^{-1} \text{ P}$  were 0.8%. A sample throughput of  $40\text{-}60 \text{ h}^{-1}$  was achieved. The proposed method was validated by analysis of certified reference materials of soil and water, and was successfully applied to real-life environmental samples as well. Amperometric detection might be a straightforward alternative to the conventional spectrophotometric phosphomolybdenum blue method. In addition, the analytical method does not suffer from interferences such as particulates and colored substances, and refractive index effect (Schlieren's effect), and can tolerate concentrations of silicate and chloride as high as  $1000 \text{ mg L}^{-1}$  and  $40 \text{ g L}^{-1}$ , respectively.

**Keywords:** Flow injection; Amperometry; Orthophosphate; Soil; Water

## 1. Introduction

Phosphorus is one of the most essential nutrients for life on earth, including phytoplankton in aquatic systems. Excessive inputs of phosphorus from domestic and industrial wastes, as well as terrestrial soils, might lead to eutrophication of lakes and coastal sea, with potential occurrence of red tides, which are often accompanied by abnormal growth of toxic algae [1]. Orthophosphate is the major form and the most bioaccessible and bioavailable phosphorus species in natural waters. The criterion for phosphorus concentrations in non-eutrophic waters is considered to be less than  $20 \text{ }\mu\text{g/L}$  of phosphorus [2]. Accurate determination of orthophosphate at the  $\mu\text{g/L}$  level in various water compartments in the biosphere is thus important for investigation of the biogeochemical cycle of phosphorus. Analytical methods for determination of varied concentration levels of orthophosphate have been summarized in a recent comprehensive review article [1]. Flow based methods including flow injection (FI) and related approaches thereof have better performance than batch methods in term of sensitivity,

reproducibility and ease of operation. The most widely used chemistry for orthophosphate determination by FI capitalizes on the phosphomolybdenum blue reaction [3-6]. Orthophosphate reacts with molybdate, in the presence of vanadate or antimony, in acidic medium yielding phosphomolybdenum yellow, which is whereupon reduced to phosphomolybdenum blue by a given reducing agents such as ascorbic acid, stannous chloride or hydrazine. However, these reducing agents are not chemically stable, and thus not suitable for unattended monitoring or long-term determination of orthophosphate in water bodies. To tackle this drawback, on-line electrochemically reduction has been proposed [7], however, the method lacks sensitivity for environmental assays and is merely applicable to beverages and wastewaters containing high orthophosphate concentrations. Sensitivity of the molybdenum blue method is also an issue as a consequence of low molar absorptivity of the molybdenum blue product ( $2 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ ) [8]. Thus, a well assembled manifold employing a sensitive and stable detector long pathlength liquid-core waveguide capillary flow cell [3], a pulseless pumping system and highly purified reagent mixtures is needed for measurements at environmentally relevant concentrations of soluble reactive phosphorus. Utilizing the ion association of phosphomolybdate with a cationic dye, e.g., malachite green (MG), a more sensitive method was proposed bearing a molar absorptivity in the order of  $1.0 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$  [8,9]. However higher concentrations of acid and molybdate than those of the phosphomolybdenum blue method were used, thereby leading to hydrolysis of organic phosphorus or precipitation of the reagent. The ion association process is slow and sensitivity of high-throughput flow injection based methods might thus not suffice. Common to all spectrophotometric procedures is the quest of tools to minimize the

impact of concomitant matrix ingredients, e.g. silicate, colored species, turbidity and refractive index (schlieren) effects on the analytical measurements. Silicate usually occurs in environmental samples at concentration levels exceeding the target analyte in several orders of magnitude and severely interferes in the heteropolyacid-based method. Tartaric acid has been proposed as a masking agent for silicate, but cannot be efficiently used at concentrations above 2 mg L<sup>-1</sup> silicate [3]. Dedicated multi-reflection [10,11] and total internal reflection flow-through cells have proven to display minimum susceptibility to the refractive index effects.

On the other hand, electrochemical detection offers the desirable features of negligible effect of refractive index, turbidity and colored species on the analytical readouts. The amperometric detection of dissolved reactive phosphorus in a flow-based format have been recently explored [12]. Yet, no thorough investigation of this reaction has been performed as of yet and problems associated with adsorption of the blue product on the electrode surface leading to irreproducible results has been identified and reported [12].

In this work, we have closely investigated the parameters affecting the flow-injection amperometric determination of orthophosphate in different concentration ranges, with special emphasis on the common interfering effects in spectrophotometric methods. Performance of the system was evaluated in the analysis of certified reference materials (CRMs) of water and soil, and determination of orthophosphate in several real-life waters and soil extracts.

## **2. Experimental**

## 2.1 Chemicals

All chemicals used were of analytical reagent grade. Deionized water from a Milli-Q, Millipore (Billerica, MA, USA) was used throughout. Acidic molybdate solution (0.5 % (w/v) in 2.5% (v/v) sulfuric acid) was prepared by dissolving 1.25 g of ammonium molybdate (Ajex Finechem, Australia) in water and 6.50 mL of concentrated sulfuric acid (Merck, Germany) was added before adjusting the final volume to 250 mL. A 0.1 mol/L potassium chloride solution was prepared by dissolving 1.86 g KCl (Merck, Germany) with water and making up to 250 mL. Orthophosphate stock solution (1000 mg P L<sup>-1</sup>) was prepared by dissolving 0.4394 g KH<sub>2</sub>PO<sub>4</sub> (Merck, Germany) with water to 100 mL. Working standard solutions within the concentration range of 20 - 1000 µg P L<sup>-1</sup> were daily prepared from an intermediate solution (5 mg P L<sup>-1</sup>) from the stock solution. Electrode polishing kit (model MF-1000, BAS, USA) was used for cleaning of the working electrode.

## 2.2 FI manifold

The FI system used is schematically depicted in Fig. 1. It consisted of a peristaltic pump (Ismatec, Switzerland), a six port injection valve (Upchurch Scientific, USA), a flow-through electrochemical cross cell (Model MF-1093, BAS, USA) embodying a 3 mm diameter glassy carbon working electrode (GCE), a stainless steel auxiliary electrode and a Ag/AgCl reference electrode containing 3 mol/L KCl). A compact potentiostat (Palmsens Vs 3.6, Netherlands) was connected to a PC for signal recording. All tubings used were of PTFE of inner diameter of 0.5 mm, except Tygon pump tubing (Saint-Gobain Performance Plastics, USA).

### *2.3 Analytical Procedure*

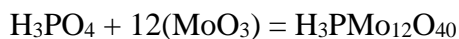
A stream of KCl solution was concurrently used as a carrier and electrolyte for amperometric detection, and the acidic molybdate solution as a reagent for 12-molybdophosphate (molybdenum yellow) complex formation. Both streams flowed at  $1.0 \text{ mL min}^{-1}$  each. A well-defined volume of standard/sample ( $75 \text{ }\mu\text{L}$ ) was injected into the carrier stream and then merged downstream with the reagent solution to evolve the 12 – molybdophosphate complex while flowing through a mixing coil (50 cm long). Electrochemically reduction of the complex at the glassy carbon electrode produced electrical current, which is directly proportional to the concentration of orthophosphate in the injected plug solution. The readouts were converted to voltage and continuously recorded as FI peaks, taking peak height as analytical measurement.

### *2.4 Sample Preparation*

Water samples were collected in polyethylene bottles and stored at below  $4^{\circ}\text{C}$  before analysis. Overall samples were filtered through a Whatman No. 5 filter upon collection. Water extracts of the soil sample ground to less than 100 mesh were obtained by shaking 2.00 g soil with 50 mL water and filtering the slurry through a Whatman No.5 filter paper according to [13].

## **3. Results and discussion**

In acidic medium, orthophosphate reacts with molybdate to evolve the so-called molybdenum yellow or 12-molybdophosphate heteropolyacid with molar absorptivity of less than  $10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ :



where  $\text{MoO}_3$  represents the molybdate being present as Mo(VI) in an acidic medium. In the presence of oxoacids, such as vanadate and antimonite, ternary heteropoly acids can occur, which feature better molar absorptivities and bathochromic shifts as compared to the heteropoly acid itself. Molybdenum yellow (and ternary heteropoly acids) can be readily reduced with the aid of a reducing agents, namely, ascorbic acid, tin(II) chloride or hydrazine, to form, via partial reduction of Mo(VI) to Mo(V), molybdenum blue, whose absorption maximum and molar absorption coefficient can be tuned upon the reducing agent selected. However, as described above, these reagents are not chemically inert and thus, a source of biased results. In this work, the electrochemically reduction of the 12-molybdophosphate was investigated in detail in a flow injection network aimed at the amperometric determination of orthophosphate. Effects of the involved physicochemical parameters on analytical method performance are presented below.

### *3.1 Investigation of applied potential on FI amperometric response*

The effect of the potential applied to GCE working electrode on the electrochemical reactions of 12-molybdophosphate was initially investigated through the use cyclic voltammetry. The reduction and oxidation peaks were obtained at about 0.19 and 0.22 V vs Ag/AgCl reference electrode, respectively. The applied potential was re-investigated in the FI amperometric system depicted in Fig. 1 exploiting the experimental

conditions given under Experimental. A Standard solution of orthophosphate ( $5.0 \text{ mg L}^{-1}$ ) and potential interfering ions at the concentrations given in Fig 2 were injected into the FI system to estimate system's response against GCE potential. A nominal potential of 0.20 V vs Ag/AgCl was selected because rendered appropriate sensitivity and stable baseline. Although the lower the potential the better was the analytical signal, a significant baseline drift was observed with the consequent deterioration of the signal to noise ratio. At 0.20 V, a silicate to phosphorus ratio of 200 was tolerated, that is,  $1000 \text{ mg L}^{-1}$  silicate at a  $5 \text{ mg/L P}$  level, yet  $5 \text{ mg L}^{-1}$  of arsenate increased the peak height of orthophosphate by 9%. Nonetheless, the concentration of arsenate in environmental samples is normally several orders of magnitude lower than that of the target analyte. Fe(III) alone did not give any appreciable amperometric FI peak, but addition of  $10 \text{ mg/L Fe(III)}$  to  $5 \text{ mg/L P}$  standard solution generated a depletion of analytical signal (see Fig. 2) 1, most likely due to precipitation of iron phosphate .

### *3.2 Effect of reagents on FI amperometric response*

The Effect of molybdate concentration on the FI readouts was investigated within the range of 0.2 – 1.0 % (w/v) at 0.20 V vs Ag/AgCl. A series of orthophosphate standard solutions ( $1.0 – 10.0 \text{ mg L}^{-1}$ ) were injected into the system at each individual molybdate concentration and a calibration linear graph was constructed. A plot of the slope of the calibration graphs versus concentration of molybdate is shown in Fig. 3. It was found that the higher the heteropolyacid-forming reagent concentration the better was the analytical sensitivity. However, we have detected that molybdate concentrations above 0.5% (w/v) rendered systematically significant baseline drift as a result of



adsorption of the generated molybdenum blue at the GCE surface, which could be observed by naked eye. A 0.5 % (w/v) molybdate concentration was selected for further investigations. The concentration of sulfuric acid in the reagent line was varied within the range of 0.0 – 3.5 % (v/v). A blank solution (deionized water) and 5 mg P L<sup>-1</sup> phosphate solution were injected in triplicate into the system at each assayed concentration of sulfuric acid. A plot of peak height versus concentration of sulfuric acid is depicted in Fig. 4. A 2.5 % (v/v) sulfuric acid was selected because sufficiently high analytical signals and stable baseline were thus recorded. Although the sensitivity of the amperometric method was improved at 2.75 and 3.0 % (v/v) sulfuric acid, a concomitant increase of baseline noise was observed which worsened method's repeatability and reproducibility.

### *3.4 Analytical characteristics*

Using the selected experimental conditions, linear calibration graphs spanned different concentration ranges, namely, 10-100 µg L<sup>-1</sup>P ( $Y = 0.0002X + 0.0005$ ,  $R^2 = 0.9995$ ), 0.1-1.0 mg L<sup>-1</sup> P ( $Y = 0.245X - 0.001$ ,  $R^2 = 0.9996$ ), and 1.0-10.0 mg L<sup>-1</sup> P ( $Y = 0.0732X + 0.0195$ ,  $R^2 = 0.9981$ ). The system yielded stable and negligible baseline for at least 2.5 h of continuous flow operation, even following injection of high concentrations of orthophosphate (see Fig. 5), thus denoting the absence of carryover effects. It should be here noted that the cleanliness of the working electrode surface and careful adjustment of the electrochemical flow cell to let the solution flow freely is necessary to prevent noisy baselines and signal drifts. The limit of detection was calculated using the 10-100 µg L<sup>-1</sup>P linear range calibration graph on the basis of  $3\sigma_{\text{blank}}$  criterion and found to be 3

$\mu\text{g P L}^{-1}$ . Relative standard deviations for 11 replicate injections of either  $0.5 \text{ mg P L}^{-1}$  or  $5 \text{ mg P L}^{-1}$  were 0.8%. A sample throughput of  $40\text{-}60 \text{ h}^{-1}$  was achieved, with a minute consumption of reagents (only ca. 1 mL each of electrolyte and reagent solutions, corresponding to 7 mg KCl, 5 mg ammonium molybdate and 0.026 mL sulfuric acid per analysis).

### *3.5 Interference study*

Some potentially interfering species occurring in environmental waters or solid substrates have been thoroughly investigated. The tolerable concentrations of different concomitant species defined as the concentration causing a change in peak height of  $0.5 \text{ mg P L}^{-1}$  of  $\leq 5\%$ ) are listed in Table 1. It is well known that both silicate and arsenate are able to form heteropoly acid complexes with molybdate alike orthophosphate, yet only arsenomolybdate is here proven to be reduced at the electrode. Unlike chemical reduction in spectrophotometric assays of dissolved reactive phosphorus wherein silicate is regarded as the most severe interference, the amperometric method herein proposed tolerates up to  $1000 \text{ mg L}^{-1}$  silicate. Sodium chloride did not interfere up to  $40 \text{ g L}^{-1}$  (4% (w/v)), indicating that this method can be directly applied to seawater samples without being influenced by refractive index (schlieren) effects.

### *3.6 Method validation and application to real samples*

Certified reference materials for soil (NIST-SRM 2711) and water (NUTRIENTS –SRM Lot.40720 – 40721) were analyzed. CRM of Soil was digested for determination of total P as orthophosphate by accurately weighing 0.50 g of the CRM followed by

addition of 10 mL water and 2 mL concentrated HCl and heating to nearly dryness. The extract was filtered through a Whatman No.40 filter paper, and made up to 50.00 mL with Milli-Q water. The analytical results compiled in Table 2, indicate a good agreement between certified values and those obtained with the proposed FI amperometric method. Since the analysis was performed without any prior sample treatment (e.g., masking) to account for the interferences, excepting a 2-fold sample dilution, the proposed flow-through amperometric method is able to tolerate a large number of concomitant species in CRMs, namely, Ag 4.63 mg/kg, Al 65300 mg/kg, As 105 mg/kg, Ba 726 mg/kg, Ca 28800 mg/kg, Cd 41.7 mg/kg, Cu 114 mg/kg, Fe 28900 mg/kg, Hg 6.25 mg/kg, K 24500 mg/kg, Mg 10500 mg/kg, Mn 638 mg/kg, Na 11400 mg/kg, Ni 20.6 mg/kg, Pb 1162 mg/kg, S 420 mg/kg, Sb 19.4 mg/kg, Se 1.52 mg/kg, Si 304400 mg/kg, Sr 245.3 mg/kg, Ti 3060 mg/kg, Tl 2.47 mg/kg, V 81.6 mg/kg, Zn 350.4 mg/kg.

The FI method was applied to analysis of soil and surface water samples (see Fig. 5) as well. Samples were prepared as described in section 2.4. The results were compared with those of the batch APHA-AWWA-WEF spectrophotometric standard method [14] based on molybdenum blue using ascorbic acid as a reducing agent and potassium antimonyl tartrate acting simultaneously as a catalyst and masking agent. According to the PAIRED t-test [15], no significant differences at the 95% confidence level were found between results obtained from both analytical methods as presented in Table 3.

#### **4. Conclusion**

An FI amperometric system was herein developed for fast and reliable determination of orthophosphate in environmental aqueous and solid substrates over the

range of 0.01-10.0 mg P L<sup>-1</sup> (in solution). The method is straightforward and merely uses a compact cost-effective potentiostat as an amperometric detector and small amounts of readily available chemicals, and features low detection limit (3 µg P L<sup>-1</sup>), with excellent precision (RSD of 0.8% for 11 replicate injections at the 0.5 and 5 mg P L<sup>-1</sup> levels).

Amperometric detection also has superior advantages over the classical molybdenum blue- based spectrophotometric method, e.g., tolerance of silicate and chloride up to 1000 mg L<sup>-1</sup> and 4% (w/v), respectively, without interference from turbidity, refractive index changes and colored substances. The developed method was successfully applied to the determination of dissolved reactive phosphorus in CRMs and real-life soil and surface waters.

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**Figure captions**

Figure 1 Schematic diagram of FI manifold for flow-through amperometric determination of orthophosphate; E = 0.1 mol/L KCl, R = 0.5% (w/v) molybdate in 2.5% (v/v) sulfuric acid, P = peristaltic pump, S = standard/sample, I = injection valve, MC = mixing coil, FC = electrochemical flow through cell, W = waste, D = potentiostat/amperometric detector, PC = personal computer.

Figure 2 Effect of GCE potential on analytical readouts, E = 0.1 mol/L KCl, R = 0.5% (w/v) molybdate in 2.5% (v/v) sulfuric acid, flow rate of each line 1.0 mL min<sup>-1</sup>.

Figure 3 Effect of concentration of molybdate on method's sensitivity; applied potential 0.20 V, E = 0.1 mol/L KCl, R = 2.5% (v/v) sulfuric acid with various concentrations of molybdate, flow rate of each line 1.0 mL min<sup>-1</sup>.

Figure 4 Effect of concentration of sulfuric acid on analytical readouts; applied potential 0.20 V, E = 0.1 mol/L KCl, R = 0.5% (w/v) molybdate in various concentrations of sulfuric acid, flow rate of each line 1.0 mL min<sup>-1</sup>.

Figure 5 FIAgram obtained in the determination of orthophosphate in environmental samples exploiting the proposed flow-through amperometric setup : applied potential 0.20 V, E = 0.1 mol/L KCl, R = 0.5% (w/v) molybdate in 2.5% (v/v) sulfuric acid, flow

rate of each line  $1.0 \text{ mL min}^{-1}$ . Values in the figure indicate concentration of orthophosphate in  $\text{mg P L}^{-1}$ .