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Sequential injection determination of orthophosphate as ion associate of 12-molybdophosphate with Astra Phloxine

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ABSTRACT

A simple and fast reaction between 12-molybdophosphate heteropoly anion and the polymethine dye Astra Phloxine was used for the development of a new SIA method for the determination of orthophosphate. The suggested method has higher sensitivity and a broader calibration range than existing SIA methods. It can be used to detect phosphate in the range from 0.2 to 10 μmol L^{-1} with a detection limit of 0.1 μmol L^{-1} and an acceptable throughput of 20 samples h^{-1}. The comparably low molybdate (5.6 mmol L^{-1}) and dye (0.1 mmol L^{-1}) concentrations led to an improvement in the stability of the base line. Inter-day reproducibility was better than 5%, while the intra-day RSD was in the range 0.8–3.5%. The method was used for the analysis of natural water samples and phosphorus-containing chemicals having a low content of orthophosphate.

1. Introduction

Molybdophosphate heteropoly blue (phosphomolybdenum blue) and various ion association complexes (IA) of basic dyes with 12-molybdophosphate heteropoly anion (12-MPA) are two commonly used analytical forms for the determination of orthophosphate. The formation of phosphomolybdenum blue has been the basis for many flow injection (FI) procedures used to determine phosphate. Despite their higher molar absorptivity, IAs have not been widely used in flow-based systems for phosphate determination [1,2], mainly because low soluble aggregates of IA can be adsorbed onto the inner walls of FI system components (PTFE tubing, joints, flow cell, etc.), leading to a drift in the baseline [3].

Usually, with the formation of an IA by a basic dye and a heteropoly anion, the spectra of the dye and its IA with the heteropolyanion (HPA) or isopolymolybdate anions used as a reagent are very close to one another. Therefore, it is necessary to separate the dye excess and its IA with isopolymolybdate ions from the IA dye–HPA. This can be done using extraction, flotation and centrifugation [4], but such procedures complicate the determination, reduce reproducibility and prolong the determination. The best alternative method is based on the discoloration of the excess of triphenylmethane dye, which occurs through sufficient acidification due to the selective protonation of only dye cations not bound in an IA with HPA [2]. Malachite green has been the most widely used reagent among other dyes [5]. Nevertheless, besides the aforementioned disadvantages, such reactions are somewhat slow [6] and can lead to high blanks.

It was recently shown that polymethine dyes are capable of forming stable IAs with HPAs [7–9]. This reaction is accompanied by a marked change in the color of the solution. Thus, the absorbance of the IA can be measured directly and without preliminary separation of the dye. The color of the IA develops immediately after mixing reagents and remains constant for several hours. The method is much more sensitive than "phosphomolybdenum blue" or "malachite green" procedures, and its selectivity is significantly better for many species. High concentrations of chloride [10] and many other ions do not interfere with the determination, and phosphate can be determined in highly salty waters such as samples from the Dead Sea [7]. The absence of additional steps, in addition to the very rapid formation of colored substance, makes the determination highly reproducible.

The formation of IA by Astra Phloxine (AP) and the reduced form of 12-MPA was used for the development of an automated procedure for the determination phosphate by cyclic-injection analysis [9]. The configuration of the flow system used allowed for negligible dispersion, a fact that explains why the molar absorptivity obtained was nearly the same as in the batch method, that is,

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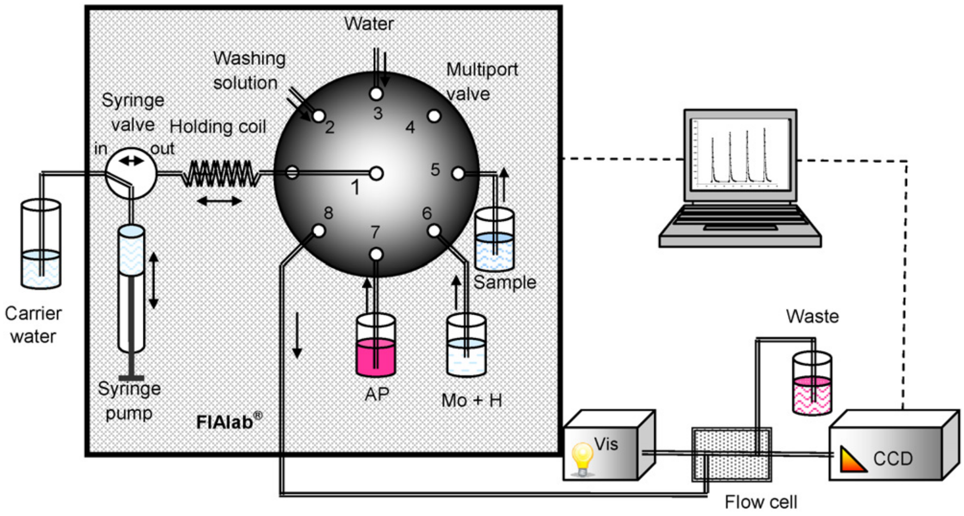

Fig. 1. Scheme of the SIA manifold for the determination of phosphate. CCD: charge-coupled detector; Vis: tungsten lump; AP: Astra Phloxine, $C_{AP} = 0.1\ \text{mmol L}^{-1}$; Mo + H: $C_{Mo(VI)} = 5.6\ \text{mmol L}^{-1}$, $C_H = 0.2\ \text{mol L}^{-1}$; holding coil length 20 cm; $\lambda = 574$ nm; flow rate $25\ \mu\text{L s}^{-1}$.

$1.6 \times 10^5\ \text{mol}^{-1}\ \text{L cm}^{-1}$. The detection limit achieved was $5\ \mu\text{g L}^{-1}$ at a sample volume of 0.3 mL. The method was distinguished by a high consumption of reagents (0.6 mL) and a slow sampling rate of 6 analyses per hour.

Sequential injection analysis (SIA) is a versatile flow method using programmable flow as well as possibility of flow redirection. Among the major benefits of SIA systems are reduced consumption of reagents, robust hardware and flexible control software, which provides convenient optimization and operation of the system.

The determination of phosphorus is very important for monitoring rivers, lakes and oceans. Increased phosphorus concentrations resulting from overfertilization or release from industrial and municipal sources cause eutrophication and other adverse effects [1]. Phosphate concentrations in oligotrophic systems can be less than $10\ \mu\text{g L}^{-1}$ [11], making them difficult to analyze using FI procedures based on the formation of phosphomolybdenum blue. Moreover, due to its slowness, the phosphomolybdenum blue method may overestimate orthophosphate by erroneously including acidic hydrolysable organic or condensed phosphorus compounds [12].

The many existing FI and SI procedures for the determination of phosphate are based on slow reactions [1,2]. Thus their use in FI systems leads to a significant increase in dispersion and a decrease in sensitivity. In this work, the very fast reaction of 12-MPA with Astra Phloxine was used to develop a simple, low-cost, sensitive and selective SIA method for the determination of orthophosphate. The applicability of the method was demonstrated through the analysis of real water samples.

2. Materials and methods

2.1. Reagents

All reagent solutions were prepared from analytical grade chemicals. Water was purified on a Milli-Q system (Millipore, USA). A phosphorus(V) stock solution containing $0.1\ \text{mol L}^{-1}$ of phosphate was prepared by dissolving KH_2PO_4 in water. A $0.1\ \text{mol L}^{-1}$ molybdate stock solution was prepared by dissolving $Na_2MoO_4{\cdot}2H_2O$ recrystallized from water–ethanol, standardizing by evaporation and weighing, and then storing in a polyethylene bottle. Astra Phloxine (Basic Red 12, 1,3,3-trimethyl-2-[3-(1,3,3-trimethyl-2-indolinylidene)propenyl]-3*H*-indolium chloride) was purchased from Hangzhou Dayangchem Co., Ltd. (China) and used without purification. A $1\ \text{mmol L}^{-1}$ Astra Phloxine stock solution was prepared by dissolving 0.444 g of AP in 10 mL of ethanol and diluting to 1000 mL with purified water. All solutions as well as the water used as a carrier were degassed in an ultrasonic bath for 20 min prior to analysis. Standard solutions of potassium pyrophosphate, adenosine 5′-triphosphate, sodium phenylphosphate, sodium tripolyphosphate, and sodium glucose-6′-phosphate were prepared directly before analysis.

2.2. Apparatus

The SIA manifold was arranged using the following equipment: a FIAlab® 3500 system (FIAlab® Instrument Systems Inc., Bellevue, USA) consisting of a syringe pump (syringe reservoir 5 mL) and a central eight-port Cheminert selection valve (Valco Instrument Co., USA).

The manifold was equipped with a USB 2000 fiber-optic charge-coupled detector (Ocean Optics Inc., Dunedin, USA) with LS-1 tungsten lamp (Ocean Optics Inc., Dunedin, USA) as a VIS light source and a micro-volume Z-flow cell of 20 mm optical path length. All manifold tubing was made of 0.75 mm i.d. polytetrafluoroethylene (PTFE), except that used for the aspiration of water as a carrier, which was 1.5 mm i.d. The whole SIA system was controlled by the latest version of FIAlab program for Windows 5.0.

2.3. SIA procedure

The SIA manifold configuration used for the determination of orthophosphate is shown in Fig. 1. The analytical cycle begins by filling the piston pump syringe with 2000 µL of the carrier solution (water), which is drawn into the syringe at a flow rate of $100\ \mu\text{L s}^{-1}$. The syringe pump outlet (two-way valve) is then set into the out position, the flow rate changed to $25\ \mu\text{L s}^{-1}$, and 80 µL of reagent ($5.6\ \text{mmol L}^{-1}$ molybdate and $0.1\ \text{mol L}^{-1}$ sulfuric acid) using port 6 of the multiport valve, 30 µL of $0.1\ \text{mmol L}^{-1}$ solution of AP (port 7), 60 µL of sample solution (port 5), and 70 µL of water (port 3) are sequentially aspirated into the holding coil. For better mixing, 40 µL of water are initially dispensed and then aspirated through port 4. The entire volume is forced at $25\ \mu\text{L s}^{-1}$ through a Z-flow cell using port 8. The spectrometer reference scan is made after a 1 s delay and then absorbance scanning begins. The response signal is measured at 574 nm. The system is washed after every five cycles by aspirating 300 µL of 50% ethanol into the holding coil through port