

Determination of Nutrients using Amperometric Detection by Flow Injection Analysis : Part(1) Phosphate in Water

Toyoaki Aoki * and Daisuke Nakano

*College of Engineering, Osaka Prefecture University, Gakuen-cho,
599-8531 Sakai, Japan*

Abstract

The concentration of phosphate in a sample solution was determined by the reduction of molybdophosphate using a glassy carbon electrode as an amperometric detector. The applied potential of the electrode was set at -100 mV vs. AgCl/Ag, at which silicate in the sample solution did not interfere in the determination of phosphate. The calibration curve for phosphate was almost linear in a concentration range of 2×10^{-7} M to 1×10^{-5} M. The detection limit for the determination procedure for phosphate, as derived from three standard deviations, was 6×10^{-8} M. The time required to analyze a single sample by the present flow injection analysis was 1.5 min. After pretreatment by persulphate digestion, total phosphorus in the sample solution could be determined by a similar analytical procedure as that for phosphate. The present method was applied to natural waters, and compared to standard colorimetric analysis of the same samples. The concentrations of phosphate and total phosphorus in natural waters were in good agreement.

Keywords: Amperometric reduction, phosphate, total phosphorus, flow injection analysis, glassy carbon electrode

1. Introduction

Phosphate is an essential nutrient and a limiting factor for phytoplankton blooms in natural waters. In recent years, freshwater red tide has arisen in lake Biwa, which is the largest lake in Japan. It is important to determine phosphate levels in natural waters in order to prevent such algal blooms.

The most widely used method for determining phosphate levels in water is the molybdenum blue method [1] which, however, requires many reagents such as antimonyl tartarate, and ascorbic acid as a reducing reagent. Fogg and Bsebsu [2] reported the determination of phosphate levels by the amperometric reduction of molybdophosphate without the use of ascorbic acid. Harden and

Nonidez [3] also reported the use of flow injection analysis (FIA) with amperometric detection. Neither amperometric method eliminated silicate interference.

Recently, Carpenter et al. [4] and Hodgson and Pletcher [5] reported the simultaneous determination of silicate and phosphate by a combined amperometric and batch method. The purpose of their work was not to develop and optimize a specific analytical procedure, rather, their aim has been to demonstrate possibilities for the selective determination of low levels of silicate and phosphate in waters using amperometric methods with microelectrodes.

In the present paper, we report the rapid amperometric determination of phosphate levels in water by FIA. This method was free of silicate interference, and was able to be applied to the determination of phosphate and total phosphorus in natural waters.

*Corresponding author

E-mail: aoki@ams.osakafu-u.ac.jp

2. Experimental

2.1. Reagents

All chemicals were analytical grade from Wako Pure Chem. (Japan). A stock solution of 0.1 M phosphate was prepared from sodium dihydrogenphosphate dihydrate. This stock solution was stored in a refrigerator and diluted to appropriate concentration when necessary. A stock solution of 0.02 M silicate was prepared from sodium metasilicate. A stock solution of 0.03 M molybdate was prepared from ammonium molybdate. The oxidizing reagent used in the determination of total phosphorus was prepared from potassium persulphate.

Working solutions were prepared daily by appropriate dilution of the stock solutions. Double-distilled water was used for the preparation of all solutions.

2.2. Flow Injection Apparatus and Procedure

A schematic diagram of the FIA used in this study is shown in Fig. 1. A sample (250 μ l) containing phosphate was injected into a stream of 2 mM sulfuric acid solution (A), then fed by a pump (P1: Analytical Pump, Dionex, USA), into a mixing coil (M1: PTFE, inner diameter (ID) 0.5 mm, length 900 mm). The mixed solution was merged with a mixed solution of 1.8 mM molybdate and 0.2 M sulfuric acid (B), which was fed by a pump (P2: Trirotar-V, Nippon Bunko, Japan) into a mixing coil (M2: PTFE, 0.5 mm ID, length 900 mm). Molybdophosphate formed in M2 was reduced by an amperometric detector (D: ECD-100, Eicom, Japan). The applied potential of the working electrode was set at -100 mV vs. AgCl/Ag. The signal from the amperometric detector was recorded on a chart recorder (R: Chromatopac C-R18, Shimadzu, Japan).

Flow rate of solution (A) and solution (B) was fixed at 0.5 ml/min. In this system, teflon tubing (PTFE, 0.5 mm ID, 1.0 mm OD) was used as the coil and line material. In the amperometric detector (D), the working electrode, the counter electrode, and the reference electrode were glassy carbon

(diameter 12 mm: WE-GC, Eicom, Japan), stainless steel (SUS-316), and AgCl/Ag, respectively.

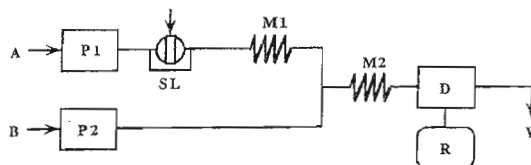


Fig.1 Schematic diagram of FIA system for determination of phosphate.

A:2mM sulfuric acid. B:0.2M sulfuric acid containing 1.8mM molybdate, P1,P2: pumps. S1:sample loop(250 μ l), M1,M2:mixing coil, D:ECD detector, R:recorder, W:waste.

3. Results and Discussion

3.1. Voltammetry in Present System

We investigated the relationship between applied potential and the reduction current of molybdophosphate in the present system. Fig. 2 shows the results obtained for a 1×10^{-5} M phosphate solution and double-distilled water as the injected sample.

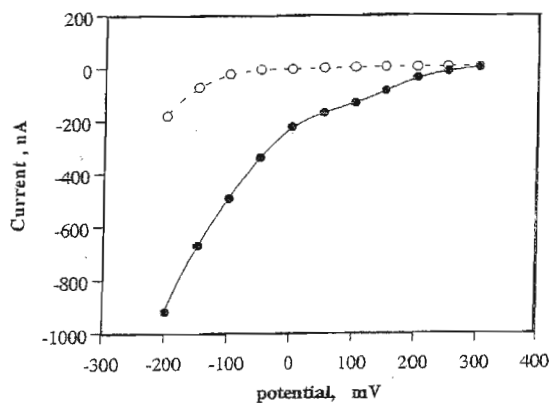


Fig.2 Relationship between applied potential (vs AgCl/Ag) and reduction current.

● : 1×10^{-5} M phosphate, ○ : water.

The reduction currents of both solutions increased as applied potential decreased. In the case of water as sample, the increase in current seems to be due to dissolved oxygen. However, when the applied potential was less

than -100 mV vs. AgCl/Ag, the current was unstable. The signal-to-noise ratios at -100, -150, and -200 mV, were 33, 12, and 6, respectively. Therefore, in the following experiments, we set -100 mV as the applied potential for the amperometric detector.

The volume of the electrolysis chamber in the amperometric detector was found to affect the stability of the signal baseline. The FIA peak height decreased with increasing volume due to diffusion of the electrode-active product, molybdo-phosphate, in the chamber. At the same time, the stability of the signal baseline increased with increasing volume due to the smoothness of the flow of solutions in the present system. As a compromise, we selected a volume of 100 μ l for the following experiments.

3.2. Optimization of Reagent Concentration

The concentration of sulfuric acid in the sample significantly influenced the determination of phosphate and silicate levels in the present system. Fig.3 shows the variation in the concentration of sulfuric acid in reagent B (from Fig. 1). In this case, the concentration of molybdate in reagent B was set at 1.8 mM. The FIA peak for silicate decreased with increasing sulfuric acid concentration. By contrast, that for phosphate increased to a maximum, and then decreased. We selected 0.2 M sulfuric acid as the optimum concentration in order to eliminate any interference from silicate in the samples.

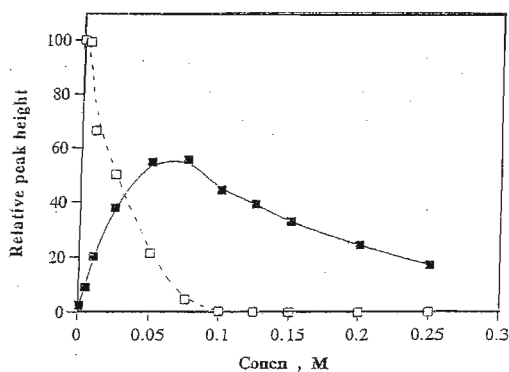


Fig.3 Effect of concn. of sulfuric acid on peak height.

■: 10^{-5} M phosphate, □: 5×10^{-5} M silicate.

Under the condition of 0.2 M sulfuric acid in reagent B, we investigated the effect of molybdate concentration. As shown in Fig.4, the FIA peak height increased with increasing molybdate concentration, up to about 3 mM molybdate, at which the peak height became relatively constant. The signal baseline became unstable at molybdate concentration exceeding 2.0 mM due to low solubility. In order to maintain the stability of the signal baseline, we selected 1.8 mM molybdate as the optimum concentration.

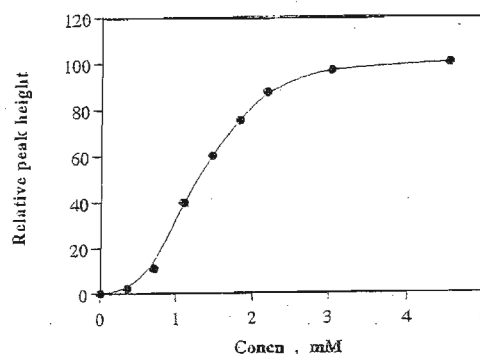


Fig.4 Effect of concn. of molybdate on peak height.

Concn. of phosphate: 1×10^{-5} M.

3.3. Interference Studies

Under the optimum conditions determined above, we investigated the interference of foreign ions, which are commonly present in fresh waters. The concentrations of foreign ions were selected as those generally found in fresh waters such as lakes and rivers. As shown in Table 1, the foreign ions investigated did not significantly interfere with the determination of phosphate levels by the present method.

The recovery of phosphate (10μ M) in the presence of silicate was studied over a silicate concentration range of 1μ M to 100μ M. Phosphate was completely recovered within measurement error, as shown in Table 2. This high rate of recovery appears to be due to the suppression of molybdosilicate formation in the strong acid. Carpenter et al.

[4] reported that silicate did not react with molybdate at pH lower than 0.43.

Table 1 Recovery of phosphate (a) in the presence of foreign ions(b)

foreign ion	recovery, %
Cl ⁻	100.4
NO ₃ ⁻	97.4
SO ₄ ²⁻	98.8
Na ⁺	101.2
NH ₄ ⁺	99.8
K ⁺	97.4
Mg ²⁺	98.8
Ca ²⁺	100.3

a) 1×10^{-5} M b) 1×10^{-6} M

Table 2 Recovery of phosphate (a) in the presence of silicate

silicate, $\times 10^{-5}$ M	recovery, %
1	101.1
5	99.7
10	100.5
50	98.8
100	100.2

a) 1×10^{-5} M

3.4. Calibration Curve and Sensitivity

The calibration curve for the determination of phosphate levels is represented in Fig.5. The figure also shows the calibration curve for the determination of total phosphorus by the present method after pretreating solutions containing known amounts of phosphate by persulphate digestion with autoclave, as a determination of total phosphorus [6]. The calibration curve for phosphate was in good agreement with that for total phosphorus. Both calibration curves are almost linear in the concentration range of 2×10^{-7} M to 1×10^{-5} M.

The detection limits for the determination

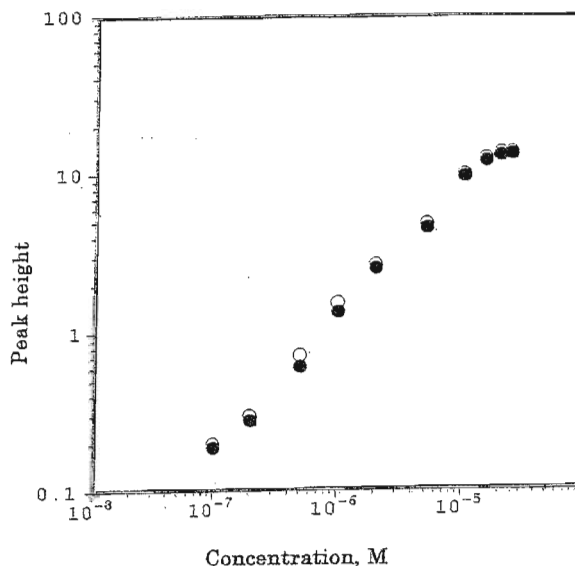


Fig. 5 Calibration curves for determination of phosphate and total phosphorus.

○ : phosphate, ● : total phosphorus.

procedures for phosphate and total phosphorus, as derived from 3σ (three standard deviations), were 6.0×10^{-8} M and 7.4×10^{-8} M, respectively. The relative standard deviations ($n=5$) at 5.0×10^{-7} M phosphate for the phosphate and total phosphorus procedures were 4.1% and 6.5%, respectively.

The time required to complete the analysis for a single sample by FIA was 1.5 min.

3.5. Application of the present method to natural waters

We applied the present method to determine phosphate levels and total phosphorus in river and lake waters. The samples ($n=16$) were taken at eight sites in the Lake Biwa and Yodo River basin on Aug.27 and Sep.30, 1998. The results for phosphate and total phosphorus are shown in Figs 6 and 7, respectively. The molybdenum blue colorimetric method [1] was applied to the same samples. Correlation coefficients between concentrations obtained by the present method and by the colorimetric method, for phosphate and total phosphorus

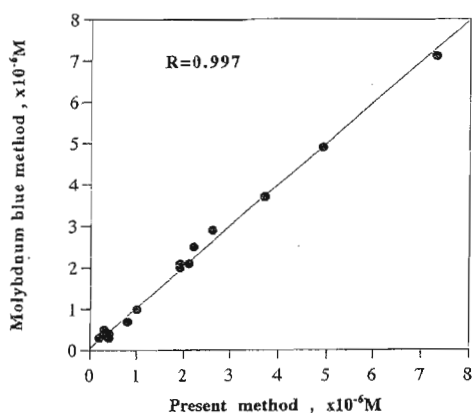


Fig.6 Correlation between the present method and molybdenum blue method for determination of phosphate in aquatic environments.

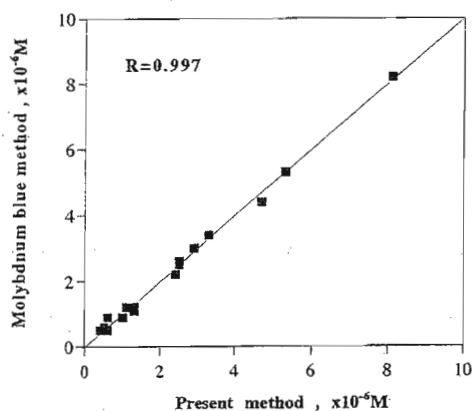


Fig.7 Correlation between the present method and molybdenum blue method for determination of total phosphorus in aquatic environments.

in all samples, were 0.997 and 0.997, respectively. Good matches were found for the concentrations determined by both methods.

4. Conclusions

Phosphate in sample solutions reacted with molybdate, producing molybdophosphate, the levels of which were determined by the reduction of molybdophosphate by a glassy carbon electrode as an amperometric detector. The applied potential of the electrode was set

at -100 mV vs. AgCl/Ag, at which silicate in sample solution did not interfere in the determination of phosphate levels under the selected conditions. The calibration curve for phosphate was almost linear in a concentration range of $2 \times 10^{-7}M$ to $1 \times 10^{-5}M$. The detection limit for phosphate using this procedure, as derived from three standard deviations, was $6 \times 10^{-8}M$. The time required to complete analysis for one sample by the present FIA method was 1.5 min. After pretreatment by persulphate digestion, total phosphorus in a sample solution could be determined by a similar analytical procedure to that for phosphate. The present method was applied to natural waters, and compared to standard colorimetric analysis of the same samples. The concentrations of phosphate and total phosphorus obtained by both methods were in good agreement.

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