

# Determination of Phosphate Utilizing Fluorescent Reaction of Thiamine with Molybdovanadophosphate by Flow Injection Analysis

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## Abstract

A fluorescence (FL) reaction between thiamine and molybdovanadophosphate was applied to a determination of phosphate by flow injection analysis (FIA). Phosphate was detected by measuring FL intensity of fluorophore produced by reaction of thiamine with molybdovanadophosphate. Under the condition of  $1.0 \times 10^{-3}$  M vanadate,  $1.8 \times 10^{-2}$  M sulfuric acid,  $1.0 \times 10^{-3}$  M molybdate, and  $7.5 \times 10^{-6}$  M thiamine, the calibration curve for the determination of phosphate was proportional in the concentration range of  $5.0 \times 10^{-7}$  M to  $2.0 \times 10^{-5}$  M. The relative standard deviations ( $n=5$ ) at  $1.0 \times 10^{-6}$  M and  $1.0 \times 10^{-5}$  M were 5.0% and 2.0%, respectively. The time required to a peak after sample injection was 2 minutes. The addition of vanadate enabled the removal of interference from silica and increased more than 50 times of sensitivity for the determination of phosphate.

**Keywords:** phosphate, FIA, fluorescence, thiamine, molybdovanadate

## Introduction

Recently, eutrophication in lake, marsh, and sea along the coast has been widely spreading due to increase in inflow of sewage, chemical fertilizer, and so on. In recent years freshwater red tide has arisen in lake Biwa. This abnormal growth of phytoplankton in aquatic environments is caused by the eutrophication.

Eutrophication is mainly caused by phosphorus-containing compounds and nitrogen-containing compounds, which are required for phytoplankton growth. The concentration of phosphorus which in natural water is generally lower than that of nitrogen, is regarded as

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limiting factor of eutrophication [1]. Therefore, it is important to determine phosphate and total phosphorus (TP) in natural waters.

J. Murphy and J. P. Riley [2] modified the molybdenum blue method to add potassium antimonyl tartrate in the reagent solution, which made the reaction time decrease from 24 hours to 10 minutes. Now their method is widely employed all over the world to determine low level of phosphate. Generally, the concentration of phosphorus in natural water is too low to investigate the cycle of chemical forms of phosphorus in detail. In order to elucidate the cycle of phosphorus in natural water, more sensitive determination method of phosphate should be developed, since phosphate is dominant in phosphorus-containing compounds.

In general, fluorimetric analysis is sensitive. J. Holzbecher and D. E. Ryan [3] reported the fluorimetric determination of phosphate by using thiamine in batch system. This method utilized the reaction that non-fluorescent thiamine was oxidized by molybdophosphate to fluorophore, thiochrome [4]. Recently, P. Linares et al. [5], applied this fluorimetric method to determine silicate by flow-injection analysis. After that, they [6] reported the fluorimetric differential-kinetic determination of silicate and phosphate in the concentration range of  $1 \times 10^{-6}$  to  $2 \times 10^{-5}$  M by flow injection analysis. They could not directly determine phosphate in the presence of silicate.

We found that molybdovanadophosphate reacts with thiamine to produce fluorophore. In this report, we report the use of molybdovanadophosphate in the determination of phosphate by a FIA method which was not interfered from silica in water and enhanced the sensitivity.

## 2. Experimental

### 2.1. Reagents

Sulfuric acid was purchased from Nakalai Tesque (Japan). All other chemicals were from Wako Pure Chem (Japan). All chemicals were analytical grade. A stock solution of 0.1M phosphate was prepared from sodium dihydrogenphosphate dihydrate. This stock solution was stored in a refrigerator and was diluted to appropriate concentration when necessary. Molybdate, vanadate, and potassium antimony tartrate solutions were prepared from ammonium molybdate, ammonium vanadate, and potassium antimony oxide tartrate hemihydrate, respectively. Doubly distilled water was used for the preparation of all solutions.

### 2.2. Flow injection apparatus and procedure

A schematic diagram for the present FIA method is shown in Figure 1. Phosphate in a sample (100 $\mu$ l) injected into stream of mixed solution of molybdate and vanadate in sulfuric

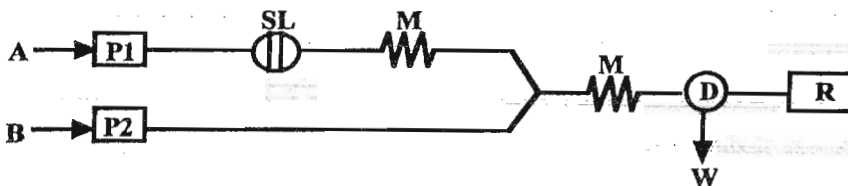


Fig. 1 Schematic diagram of a flow system for determination of phosphate A; sulfuric acid containing vanadate and molybdate, B; boric buffer solution containing thiamine, P1, P2; pumps, SL; sample loop(100  $\mu$  l), M; mixing coil, D; FL detector, R; recorder, W; waste.

acid from pump (P1) (Analytical Pump, Dionex, U.S.A.) reacted with molybdovanadate at mixing coil (M) to convert to molybdovanadophosphate. This solution was merged with thiamine in borax buffer solution (B) from pump (P2) (Trirotar-V, Nippon Bunko, Japan), and oxidized thiamine to fluorophore, thiochrome. The FL emission was detected with a FL spectrophotometer (S-3350, Soma, Japan) whose source was a xenon lamp. FL intensity was measured at 440nm using an excitation wavelength of 375nm [3]. The signal from the FL detector was recorded on a chart recorder (R: Chromatopac C-R18, Shimadzu). In this system, teflon tubing(0.5mm i.d., 1.0mm o.d.) was used as coil and line material.

### 3. Result and discussion

#### 3.1. Optimization of FL system

The ratio of the intensity of sample to blank fluorescence is important since the ratio of sample to blank (S/B) is a measure of the practical sensitivity of the method. Phosphate solution at  $1.0 \times 10^{-5}$  M was used as a sample to determine optimum conditions described below.

The effect of concentration of vanadate on FL intensity was studied over the range of  $0-2.5 \times 10^{-3}$  M. The concentrations of sulfuric acid, molybdate, and thiamine were set at 0.02M,  $2.5 \times 10^{-3}$  M, and  $1.0 \times 10^{-5}$  M, respectively. The maximal S/B was obtained at  $1.0 \times 10^{-3}$  M.

The optimum concentration of sulfuric acid on FL intensity was studied over the range of 0.012-0.024M. In this case, the concentrations of molybdate and thiamine were set at  $2.5 \times 10^{-3}$  M and  $1.0 \times 10^{-5}$  M, respectively. The maximal S/B was obtained at 0.018 M as shown in Figure 2. When the acid concentration is too low, a yellow molybdenum complex [7,8] or a precipitate [9,10] forms and interferes with the fluorometric measurement. When the acid concentration is too high, the formation of molybdovanado-phosphate development

is incomplete [7,10,11].

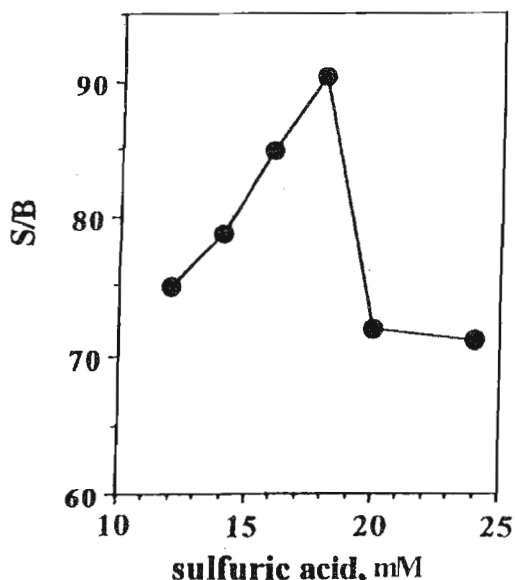
The optimum concentration of molybdate on FL intensity was studied over the range of  $0.5\text{-}5.0 \times 10^{-3}$  M under the condition where the concentration of thiamine was set at  $1.0 \times 10^{-6}$  M. The maximal S/B was obtained at  $1.0 \times 10^{-3}$  M as shown in Figure 3.

The thiamine concentration was another important variable. The maximal S/B was obtained at  $7.5 \times 10^{-6}$  M in the range of  $2.5\text{-}20 \times 10^{-6}$  M thiamine as shown in Figure 4.

Above all, in the subsequent experiments the concentrations of vanadate, sulfuric acid, molybdate, and thiamine were adjusted to  $1.0 \times 10^{-3}$  M,  $1.8 \times 10^{-2}$  M,  $1.0 \times 10^{-3}$  M, and  $7.5 \times 10^{-6}$  M, respectively.

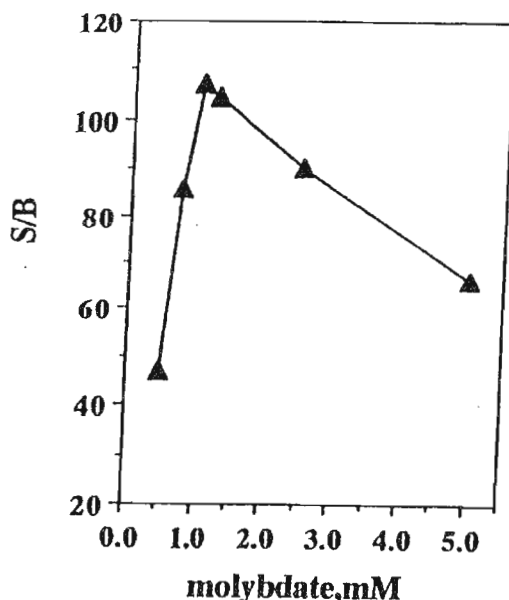
Finally, optical flow rate was determined. The total flow rate was varied over the range of 0.9-1.8ml/min. Molybdate in 0.018M sulfuric acid and thiamine in 0.1M boric buffer solution were fed up to the present system with pumps(P1,P2) as shown in Figure 1. The ratio of flow rate of molybdate solution to thiamine solution was kept at 1:2. The maximal S/B was obtained at 1.5ml/min of total flow rate, when the FL intensities were stable. Therefore, the flow rate of molybdate solution and thiamine solution were set at 0.5ml/min and 1.0ml/min, respectively.

It was reported that molybdophosphate oxidizes thiamine to fluorophore, thiochrome [4]. The fluorescent emission in the present system also is supposed to be derived from thiochrome. The fluorescence emission spectrum of thiochrome was in accord with that of the product from thiamine oxidized by molybdo-vanadophosphate.



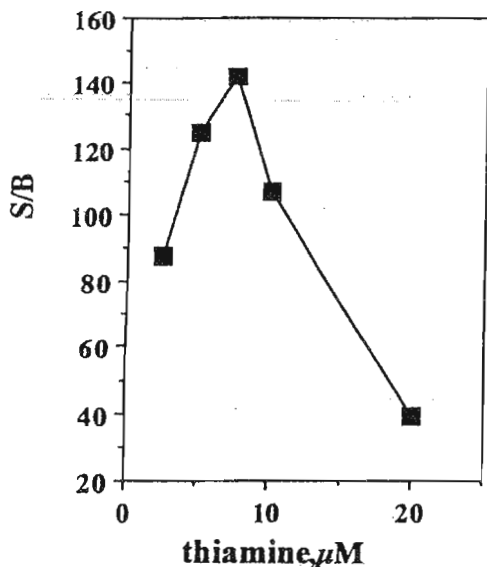
**Fig. 2 Effect of concn. of sulfuric acid on S/B**

The other experimental conditions are described in the text.



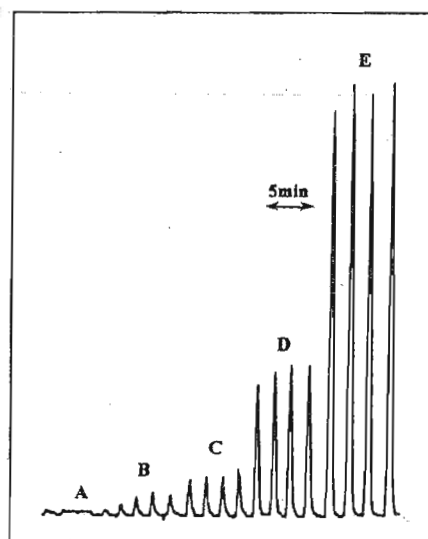
**Fig. 3 Effect of concn. of molybdate on S/B**

The other experimental conditions are described in the text.



**Fig. 4** Effect of concn. of thiamine on S/B

The other experimental conditions are described in the text.



**Fig. 5** Representative FIA peaks by present method

Concn. of phosphate : A, 0; B, 1; C, 2; D, 5 and; E,  $10 \times 10^{-6} \text{M}$ .

Therefore, it was concluded that molybdovanadophosphate oxidized thiamine to thiochrome.

### 3.2. Calibration curve and sensitivity

Under the optimum condition as described above, the calibration curve was proportional in the concentration of  $5.0 \times 10^{-7} \text{M}$  to  $2.0 \times 10^{-5} \text{M}$ . The detection limit derived from  $3\sigma$  (standard deviation) was  $2.0 \times 10^{-7} \text{M}$  in repeatative measurements of  $5.0 \times 10^{-7} \text{M}$  phosphate. The relative standard deviations ( $n=5$ ) at  $1.0 \times 10^{-6} \text{M}$  and  $1.0 \times 10^{-5} \text{M}$  were 5.0% and 2.0%, respectively. The time required for the analysis of one sample was 2 mins. Typical peak profiles for different concentrations of phosphate are shown in Figure 5.

### 3.3. Interference from foreign ions

Effect of foreign ions were shown in Table 1. We selected usual concentrations in the basin of Yodo River as concentrations of the foreign ions. The investigated foreign ions did not significantly interfered with the determination of phosphate.

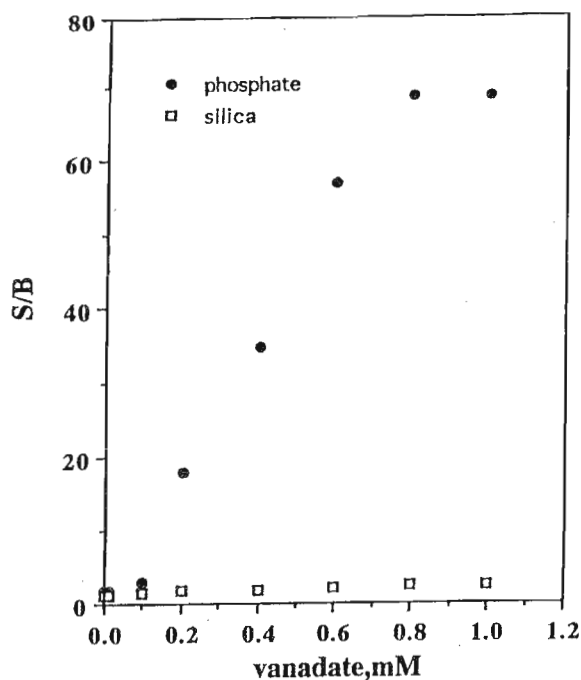
The recovery of phosphate in the presence of silica was studied over the silica concentration range of  $1.0 \times 10^{-6} \text{M}$  to  $1.0 \times 10^{-4} \text{M}$ . Phosphate was completely recovered within error of measurement. Moreover, the addition of vanadate enabled the removal of the interference from silica as well as an increase in sensitivity for phosphate as shown in Figure 6.

Table 1. Recovery of phosphate<sup>a)</sup> in the presence of foreign ions

Foreign ion	Recovery, %	Foreign ion	Recovery, %
chloride	101.8 <sup>b)</sup>	calcium	96.3 <sup>b)</sup>
nitrate	97.3 <sup>b)</sup>	magnesium	101.2 <sup>b)</sup>
sulfate	102.5 <sup>b)</sup>	iron(II)	102.7 <sup>c)</sup>
ammonium	102.9 <sup>b)</sup>	iron(III)	101.8 <sup>c)</sup>
sodium	100.5 <sup>b)</sup>	copper(II)	97.9 <sup>c)</sup>
potassium	99.5 <sup>b)</sup>		

a)  $1 \times 10^{-5} \text{ M}$ , b)  $1 \times 10^{-4} \text{ M}$ , c)  $1 \times 10^{-7} \text{ M}$ .

which demonstrated the effect of vanadate on S/B at  $1.0 \times 10^{-5} \text{ M}$  of phosphate and at  $1.0 \times 10^{-4} \text{ M}$  of silica. With the increase in concentration of vanadate, the S/B value of phosphate increased and that of silica did not. The addition of  $1.0 \times 10^{-3} \text{ M}$  vanadate increased more than 50 times of the sensitivity for the determination of phosphate. Therefore, it is supposed that the addition of vanadate makes the phosphate complex with molybdate stable.



**Fig. 6** Effect of vanadate on S/B of phosphate and silica

concn. of phosphate:  $1.0 \times 10^{-5} \text{ M}$ ; concn. of silica:  $1.0 \times 10^{-4} \text{ M}$ .

#### 4. Application the present method to natural water

We applied this method to determine phosphate in lake and river waters. The samples of river waters were taken at several sites in the basin of Yodo River. The results obtained are shown in Table 2. The colorimetric method [2] was applied to the same samples. A good match was found for the concentrations determined by both methods.

Table 2. Determination of phosphate in river and lake waters<sup>a)</sup>

Sampling site	concn. of phosphate, $\mu\text{M}$	
	Present method	Mo blue method
Hira (Lake Biwa)	0.5	0.5
Sakamoto (Lake Biwa)	0.5	0.4
Ishiyama (R. Seta)	0.8	0.5
Uji (R. Uji)	0.8	0.5
Yawata (R. Uji)	6.6	6.7
Yawata (R. Katsura)	9.6	9.8
Hirakata (R. Yodo)	7.3	7.6
Kema (R. Yodo)	2.3	2.6
Sakurajima (The mouth of R. Yodo)	2.6	2.4

a) sampling date: Dec. 27, 1997.

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