

## DETERMINATION OF TRACE PHOSPHATE ION IN WATER BY VISUAL METHOD AFTER PRECONCENTRATION ON A MEMBRANE FILTER FOR FIELD WORK

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**Summary**—A sensitive visual method based on comparing the color intensity of precipitate as phosphomolybdenum blue on a membrane filter, has been developed for the determination of trace phosphate ion in water for field work. A sample solution containing 0.05–5.0  $\mu\text{g}$  of phosphate was treated in a 25-ml polypropylene syringe, and the resulting precipitate was filtered through a membrane filter attached to the syringe. The color intensity of the precipitate on the membrane filter was measured visually following the standard series method. The coefficient of variation for five measurements at 0.5  $\mu\text{g}$  of phosphate is 11%. The detection limit is 0.02  $\mu\text{g}$  of phosphate ion in 22.5 ml (0.9  $\mu\text{g PO}_4^{3-}/\text{l}$ ) of water sample when the effective filtration area is 0.78  $\text{cm}^2$ . The interference of various ions was studied and optimum conditions were developed for the determination of phosphate ion in natural water.

It has become very important to determine trace amounts of phosphate ion *in situ* in environmental samples, especially in natural water.

There are some semi-quantitative or visual methods for field work, for example a Hach method (Hach Co., USA) and Ion Selective Pack Test (Kyoritsu Rikagaku Lab., Japan) based on the formation of phosphomolybdenum blue species in aqueous solution. These procedures have almost the same detection limit of 90  $\mu\text{g PO}_4^{3-}/\text{l}$ . And a simple visual method, based on the observation of a neutral color of malachite green ( $\text{MG}^+$ ) in a test tube with a high molar absorptivity by the ion association of  $\text{HMG}^{2+}$  and phosphomolybdate,  $\text{H}_3\text{PMo}_{12}\text{O}_{40}$  in acidic medium, has been proposed (determination limit: 30  $\mu\text{g PO}_4^{3-}/\text{l}$ ).<sup>1</sup> Further example, Environmental Protection Agency (USA) has recently validated a method for the determination of ortho-phosphate in estuarine and coastal waters. This is an automated colorimetric procedure in which the blue color of the phosphomolybdate complex is the measured species. This method has a detection limit of 1.8  $\mu\text{g PO}_4^{3-}/\text{l}$ .

On the other hand, solid phase methods based on the spectrophotometrical measurement of color intensity of membrane filter<sup>2</sup> (determination limit: 3  $\mu\text{g PO}_4^{3-}/\text{l}$ ) and ion-exchange resin,<sup>3,4</sup> (literature 3: determination limit; 3  $\mu\text{g}$

$\text{PO}_4^{3-}/\text{l}$ , literature 4: detection limit; 1.2  $\mu\text{g PO}_4^{3-}/\text{l}$ ) which were colored by adsorption of phosphate ion as phosphomolybdenum blue in natural water, have been reported. However, it is difficult to apply the methods described above to the determination of phosphate ion at several  $\mu\text{g/l}$  levels in water samples such as rain and snow for field work.

Recently, a simple and rapid preconcentration technique, based on collecting a precipitate on a membrane filter and dissolving the filter and precipitate in an organic solvent, has been reported for trace phosphate ion as phosphomolybdate by spectrophotometry,<sup>5-10</sup> fluorophotometry<sup>11</sup> and capillary isotachopheresis.<sup>12</sup> In previous studies, this preconcentration technique has been applied by the authors to the spectrophotometric determination of sulfate ion at  $\mu\text{g/l}$  levels, which is precipitated with 2-aminoperimidine and resulting compound is dissolved in nitric acid, made alkaline with sodium hydroxide and then adsorbed on tetradecyldimethylbenzyl ammonium nitrate, in rain and snow.<sup>13</sup>

In the present study, the authors developed a sensitive visual method for trace phosphate ion in water samples for field work, comparing the color intensity of a precipitate as ion-associate of phosphomolybdenum blue anion and quaternary ammonium cation on a membrane filter.

The proposed method has a wide range of determination limits.

## EXPERIMENTAL

### Apparatus

A Hitachi model 228 double-beam spectrophotometer was used to obtain absorption spectra with 10-mm pathlength micro quartz cells. A polypropylene syringe (Terumo, 25 ml) was used for filtration of a precipitate with a filter holder (Millipore Swinnex, 13 mm in diameter, effective filtration area 0.78 cm<sup>2</sup>) as shown in Fig. 1. The filter papers used were polyethersulfone membrane filter (Advantec Toyo, 13 mm in diameter, pore size 0.8- $\mu$ m) and quantitative filter papers (Advantec Toyo, No. 5C).

### Reagents

All reagents were of analytical grade, and high purity water (Millipore, Milli-QII) was used. A phosphate stock solution (1000  $\mu$ g/ml) was prepared by dissolving 0.3581 g of dried potassium dihydrogenphosphate in water and diluting the solution accurately to 250 ml; working solution were freshly prepared by dilution of the stock solution. Ammonium molybdate solution ( $9.7 \times 10^{-3}M$ ) was prepared by dissolving 3.0 g of ammonium molybdate tetrahydrate and 0.12 g of antimony(III) potassium tartrate in 250 ml of 0.15M sulfuric acid solution. L-ascorbic acid ( $8.5 \times 10^{-2}M$ ) and *n*-dode-

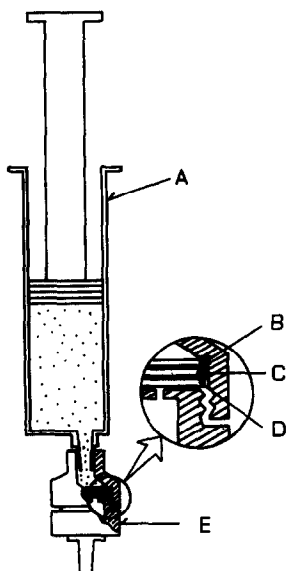


Fig. 1. Separation system: A, polypropylene syringe; B, Teflon O-ring; C, membrane filter; D, fiber filter; E, filter holder.

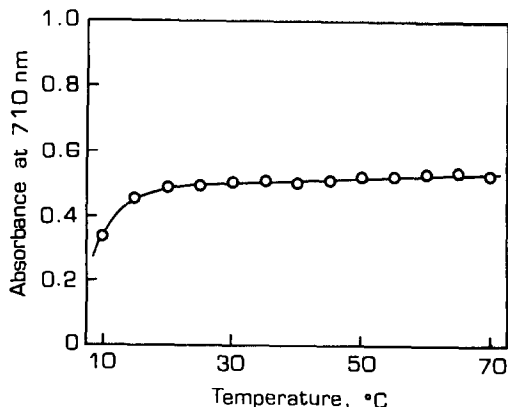


Fig. 2. Effect of reaction temperature for reduction to phosphomolybdenum blue in aqueous solution.  $PO_4^{3-}$  taken, 5  $\mu$ g.

cytrimethylammonium bromide ( $C_{12}$ TMAB,  $5.2 \times 10^{-3}M$ ) solution were prepared with water. Reducing agent was prepared fresh daily by mixing 20 ml of 1.75M sulfuric acid, 40 ml of 14% sodium metabisulfite solution and 40 ml of 1.4% sodium thiosulfate solution.

### Procedure

A sample solution containing 0.05–5.0  $\mu$ g of phosphate and 3 ml of reducing agent was drawn into a 25-ml polypropylene syringe by pulling out the plunger, and diluted to 22.5 ml with water. Then, 1 ml of  $9.7 \times 10^{-3}M$  ammonium molybdate solution and 1 ml of  $8.5 \times 10^{-2}M$  L-ascorbic acid solution were added by pulling out the plunger. The nozzle of the syringe was covered with a silicone rubber cap. The syringe was set aside for 5 min in a thermos bottle at 50°C. The cap was taken off, 0.5 ml of  $5.2 \times 10^{-3}M$   $C_{12}$  TMAB solution was added by pulling out the plunger, and the solution was shaken well. A filter holder with a membrane filter paper was attached to the syringe, and the precipitate in the solution was filtered off. The filter holder was removed from the syringe, about 5 ml of 0.15M sulfuric acid solution was drawn into the syringe. The filter holder was attached again, and the precipitate on a membrane filter paper was washed with the sulfuric acid solution by pushing the plunger. The same operation was repeated three times. Then, the washing with water was repeated three times in much the same way as with the sulfuric acid solution. The color intensity was measured visually following a standard series method.

Table 1. Effect of foreign ions

Ions	Taken* ( $\mu\text{g}$ )	Absorbance (at 710 nm)	Error (%)
None	—	0.521	—
$\text{SiO}_3^{2-}$	750	0.526	+1
	1250	0.549	+5
	2500	0.562	+8
$\text{AsO}_2^-$	15	0.530	+2
	50	0.536	+4
$\text{AsO}_3^-$	0.8	0.560	+8
	1.5	0.615	+18
	5	0.799	+53
$\text{AsO}_3^+ \dagger$	5	0.523	+1
	8	0.524	+2
	13	0.537	+4
	25	0.552	+7

\* $\text{PO}_4^{3-}$  taken = 5  $\mu\text{g}$ .

†In the presence of 3 ml of reducing agent.

## RESULTS AND DISCUSSION

First, the optimum conditions for the determination of phosphate ion were studied with accuracy by spectrophotometry based on dissolving the precipitate and membrane filter in 2 ml of dimethyl sulfoxide (DMSO). It has been confirmed experimentally in advance that visible color intensity of the precipitate on a membrane filter has good relation to the absorbance of phosphomolybdenum blue in DMSO at 710 nm.

### Absorption spectra

Phosphomolybdenum blue in DMSO has two absorption maxima at 710 and 880 nm. Beer's law is obeyed for 1.0–5.0  $\mu\text{g}$  of phosphate at both wavelengths. The molar absorptivities were  $2.0 \times 10^4$  and  $2.2 \times 10^4$  l/mol/cm and the

colors of the complex in DMSO were stable for 24 hr and 2 hr, respectively. The first peak was chosen for use, in subsequent works.

### Concentration of reagents

The effect of the concentration of ammonium molybdate solution and L-ascorbic acid solution was investigated. For a sample containing 5.0  $\mu\text{g}$  of phosphate, the optimum concentration ranges of ammonium molybdate and sulfuric acid were  $2.0 - 5.5 \times 10^{-4} M$  and 0.1–0.2M, respectively. A constant and maximum absorbance was obtained when the concentration of L-ascorbic acid was above  $1.7 \times 10^{-3} M$ . Therefore, 1 ml of  $9.7 \times 10^{-3} M$  ammonium molybdate solution and 1 ml of  $8.5 \times 10^{-2} M$  L-ascorbic acid solution were used, and 0.15M was chosen as the concentration of sulfuric acid. And then, the precipitate was washed with 0.15M sulfuric acid to remove the excess of reagents.

### Effect of reaction temperature

Effect of reaction temperature for reduction of phosphate ion to phosphomolybdenum blue in aqueous solution was investigated. A suitable temperature was about 40°C as shown in Fig. 2. The absorbance at 50°C was 2.5% greater than the one at 40°C. To heat the sample solution to about 5–10°C until  $40 \pm 1^\circ\text{C}$  in a 25-ml syringe, it took 5 min in a thermos filled with 50°C water. Though the absorbance increased gradually as the reaction time proceeded, the rate of increase after 1 hr was about 4% at 40°C.

### Filtration and washing

Although any membrane filter with pore sizes  $< 0.8 \mu\text{m}$  can be used,  $0.8 \mu\text{m}$  are recommended because of their higher filtration rate. A fiber filter paper was placed on the filter support screen to prevent the precipitate from spreading unevenly on a membrane filter as shown in Fig. 1. The precipitate on a membrane filter should be washed three times with about 5 ml of 0.15M sulfuric acid solution and three times with water, because the reagent blank appears blue slightly in the presence of L-ascorbic acid and sulfuric acid. The color intensity of the precipitate washed with acid and water was stable for half a year in the dark.

Table 2. Analysis of water samples

Sample*	pH	Electric conductivity ( $\mu\text{S}/\text{cm}$ )	$\text{PO}_4^{3-}$ added, ( $\mu\text{g}$ )	$\text{PO}_4^{3-}$ found, ( $\mu\text{g}$ )
Snow	4.50	25.0	None	0.05
			None†	0.05
			0.4	0.5, 0.4
Rain	4.47	21.5	None	0.1
			None†	0.1
			0.4	0.5, 0.5
River water	3.67	146.7	None	0.5
			None†	0.2
			0.3	0.6, 0.8
			0.3†	0.4, 0.5
Sea water	8.14	$4 \times 10^4$	None	0.5
			None†	0.5
			0.3	0.8, 0.8

\*Sample volume taken = 10.0 ml.

†In the presence of 3 ml of reducing agent.

*Effect of other ions*

The effects of commonly occurring foreign ions have been investigated.<sup>6</sup> In this study, the tolerance limit was set as the amount which caused an error of  $\pm 5\%$  in the recovery of 5  $\mu\text{g}$  of phosphate ion. At this phosphate ion level, concentrations of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  below 300 mg and  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{F}^-$ ,  $\text{Br}^-$  and  $\text{I}^-$  below 1 mg did not interfere. Silicate, arsenate and germanate also form phosphomolybdenum blue anions. Germanate is not likely to be present in concentrations high enough to cause significant interference in water samples. Arsenate causes large positive errors, but this interference can be eliminated by reducing to arsenite<sup>14</sup> as shown in Table 1.

*Series of standards*

The color intensity of the precipitate on a membrane filter was measured visually following the standard series method. The amounts of standard phosphate distinguished their color intensity was 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.40, 0.50, 0.60, 0.80, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0  $\mu\text{g}$  as  $\text{PO}_4^{3-}$ . The coefficient of variation for five measurements (0.5, 0.4, 0.6, 0.5 and 0.6  $\mu\text{g}$ ) at 0.5  $\mu\text{g}$  of phosphate ion is 11%. The detection limit is 0.02  $\mu\text{g}$  of phosphate in 22.5 ml of sample solution.

*Application to natural water samples*

The proposed method was applied to the analysis of water samples. The results are shown in Table 2.

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## REFERENCES

1. H. Hashitani and M. Okumura, *Fresenius' Z. Anal. Chem.*, 1987, **328**, 251.
2. K. Hiuro, T. Tanaka, A. Kawakura, M. Adachi and K. Fukushi, *Bunseki Kagaku*, 1982, **31**, E401.
3. K. Matsuhisa, K. Ohzeki and T. Kambara, *Bull. Chem. Soc. Japan*, 1982, **55**, 3335.
4. J. M. Bosque-Sendra, C. Merida and L. F. Capitan-Vallvey, *Analisis*, 1990, **18**, 531.
5. S. Taguchi, E. Ito-oka and K. Goto, *Bunseki Kagaku*, 1984, **33**, 453.
6. S. Taguchi, E. Ito-oka, K. Masuyama, I. Kasahara and K. Goto, *Talanta*, 1985, **32**, 391.
7. C. Matsubara, Y. Yamamoto, G. Odaka and K. Takamura, *Bunseki Kagaku*, 1987, **36**, 189.
8. C. Matsubara, Y. Yamamoto and K. Takamura, *Analyst*, 1987, **112**, 1257.
9. C. Matsubara, K. Yoshihara, N. Kobayashi, Y. Yonehara and K. Takamura, *Bunseki Kagaku*, 1989, **38**, 627.
10. M. Taga and M. Kan, *Bull. Chem. Soc. Jpn.*, 1989, **62**, 1482.
11. M. Kan, T. Nasu and M. Taga, *Anal. Sci.*, 1991, **7**, 87.
12. M. Taga, M. Kan, F. Komatsu, S. Tanaka and H. Yoshida, *Anal. Sci.*, 1989, **5**, 219.
13. J. Shida, H. Satake, N. Ono and T. Fujikura, *Talanta*, 1990, **37**, 633.
14. J. C. van Schouwenburg and I. Walinga, *Anal. Chim. Acta*, 1967, **37**, 271.