The Spectrophotometric Determination of Phosphate Following the Collection of Ion Pair of Molybdophosphate with Bis[2-(5-chloro-2-pyridylazo)-5-diethylaminophenolato]cobalt(III) on an Organic Solvent-Soluble Membrane Filter

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A rapid and sensitive method of determining phosphate was described. The ion pair of molybdophosphate with bis[2-(5-chloro-2-pyridylazo)-5-diethylaminophenolato]cobalt(III) (Co-5-Cl-PADAP) formed in 0.075 mol dm^{-3} sulfuric acid, 0.225 mmol dm^{-3} ammonium molybdate, and 25 μmol dm^{-3} Co-5-Cl-PADAP was collected on an organic solvent-soluble membrane filter made of cellulose nitrate. The membrane filter with the ion pair was then dissolved into *N,N*-dimethylformamide, and the absorbance of the solution was measured for the determination of phosphate. The calibration curve was linear up to 0.4 μg as phosphorus, with the correlation coefficient of 0.999, and the apparent molar absorption coefficient of phosphate was 2.9×10^5 dm^3 mol^{-1} cm^{-1}. The relative standard deviation was 1.7% for 0.4 μg of phosphorus. Although dodecyl sulfate existing in an amount ten times that of phosphorus caused a positive error, no ions commonly existing in natural waters interfere with the determination of phosphate. The proposed method was applied to the determination of μg dm^{-3} levels of phosphate-phosphorus in natural waters, and satisfactory results were obtained.

Since the determination of traces of phosphate in environmental samples is important for environmental chemistry and geochemistry, the development of a sensitive and rapid method of determining phosphate has been promoted. In addition to conventional heteropoly blue methods, many more sensitive methods have been reported, such as spectrophotometry using Malachite Green[1,2] and differential-pulse anodic voltammetry.[3]

For the determination of traces of phosphate in natural waters, an extraction procedure using a separating funnel is required in most determination methods. For the rapid and sensitive determination of phosphate and silicate, the collection of the ion pair of molybdophosphate or molybdosilicate with a cationic surfactant on an organic solvent–soluble membrane filter has been reported.[4–6] These methods are superior to the extraction method using a separating funnel because there is no use of toxic and combustible solvents and the operation is easy. However, greater sensitivity is required for the determination of traces of phosphate in natural waters.

For the highly sensitive determination of phosphate with membrane-filter collection, the use of Malachite Green as the ion pair reagent has been reported.[7] Although this method had a high sensitivity, the interferences caused by silicon and iron(III) were so serious that pretreatment was required for the determination of phosphate in natural waters.

In their previous studies, the present authors have developed highly sensitive methods of determining phosphate by means of indirect atomic absorption spectrophotometry using the copper(II)-1,10-phenanthroline complex[8] and have also developed flotation-spectrophotometry[9] and indirect atomic absorption spectrophotometry[10] using bis[2-(5-chloro-2-pyridylazo)-5-diethylaminophenolato]cobalt(III) chloride (Co-5-Cl-PADAP), which has previously been used for the extraction-spectrophotometric determination of anionic surfactants.[11,12]

In the present paper, the present authors have developed a highly sensitive and rapid method of determining phosphate. In the acidic solution, phosphate forms molybdophosphate with molybdate. The ion pair of the molybdophosphate anion with the Co-5-Cl-PADAP cation is formed and collected on the organic solvent-soluble membrane filter made of cellulose nitrate. The membrane filter with the ion pair is dissolved into *N,N*-dimethylformamide (DMF). The absorbance of the solution is proportional to the concentration of phosphate. By using this procedure, traces of phosphate in natural waters can easily be determined.

Experimental

Reagents. A standard solution of phosphate was prepared by dissolving potassium dihydrogenphosphate (special grade, Wako Pure Chemical Industries, Ltd.) in water. The Co-5-Cl-PADAP was purchased from Dojindo Laboratories and used as an aqueous solution without any further purification.

Apparatus. A Hitachi Model 124 double-beam spectrophotometer was used for measuring the absorbance. The absorption spectra were constructed with a Hitachi Model U-3200 recording spectrophotometer.

Procedure. A sample is placed in a 20-cm^3 beaker, and 1-cm^3 portions of 0.75 mol dm^{-3} sulfuric acid, 2.25 mmol dm^{-3} ammonium molybdate, and 0.25 mmol dm^{-3} Co-5-Cl-PADAP are added, after which the total volume is made up to 10 cm^3 with water. The solution is filtered through a 3.0-μm-pore sized membrane filter made of cellulose nitrate 25 mm in diameter (Toyo Roshi Kaisha, Ltd.). After washing with two portions of 10 cm^3 of water, the membrane filter with the ion pair is transferred into a 10-cm^3 beaker. By the addition of 5.0 cm^3 of DMF, both the ion pair and the membrane filter are dissolved. The absorbance of the solution is measured at the absorption maximum wavelength of 560 nm against water with a 1-cm measuring cell.

Results and Discussion

Absorption Spectra. The absorption spectra of the ion pair of molybdophosphate with Co-5-Cl-PADAP and a reagent blank are shown in Fig. 1. The maximum absorbance is observed at the wavelength of 560 nm.

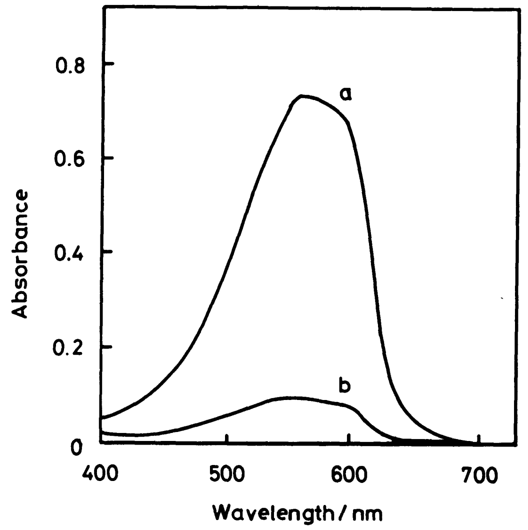

Fig. 1. Absorption spectra of ion pair of Co-5-Cl-PADAP with molybdophosphate and reagent blank. a: 0.4 μg phosphorus against reagent blank, b: reagent blank against water. [H_2SO_4]: 0.075 mol dm^{-3}, [$(NH_4)_6Mo_7O_{24}$]: 0.225 mmol dm^{-3}, [Co-5-Cl-PADAP]: 25 μmol dm^{-3}.

Selection of Acid. For adjusting the concentration of acid, sulfuric acid and hydrochloric acid can be used because of the small absorbance of the blank. The use of nitric acid or perchloric acid leads to a large absorbance of the blank due to the formation of the ion pair of nitrate or perchlorate with Co-5-Cl-PADAP. In the proposed method, sulfuric acid is used because the preservation and pretreatment needed for the determination of total phosphorus in natural waters often require the use of sulfuric acid.[13,14]

Effect of Concentration of Sulfuric Acid. Figure 2 shows the effect of the concentration of sulfuric acid on the absorbance of the ion pair and the reagent blank. The absorbance of the reagent blank was small above the concentrations of 0.05 mol dm^{-3}. The maximum absorbance of the ion pair was obtained in the concentration range from 0.05 to 0.1 mol dm^{-3}. The reason for the large absorbance of the reagent blank in the concentrations below 0.05 mol dm^{-3} was the formation and collection of the ion pair of Co-5-Cl-PADAP with molybdate. No molybdophosphate was formed in the concentration range above 0.2 mol dm^{-3}. The concentration of 0.075 mol dm^{-3} was chosen as the concentration of sulfuric acid.

Effect of Concentration of Ammonium Molybdate. The effect of the concentration of ammonium molybdate is shown in Fig. 3. The maximum and constant absorbance of the ion pair was obtained in the concentration range above 0.15 mmol dm^{-3}. As the concentration of ammonium molybdate, 0.225 mmol dm^{-3} was chosen.

Effect of Concentration of Co-5-Cl-PADAP. Figure 4 shows the effect of the concentration of Co-5-Cl-PADAP on the absorbance. The maximum and constant absorbance of the ion pair was obtained

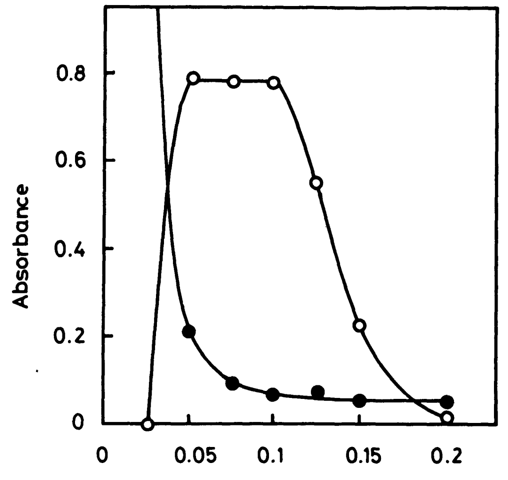

Fig. 2. Effect of concentration of sulfuric acid on absorbance. [$(NH_4)_6Mo_7O_{24}$]: 0.225 mmol dm^{-3}, [Co-5-Cl-PADAP]: 25 μmol dm^{-3}. ○: 0.4 μg phosphorus against reagent blank, ●: reagent blank against water.

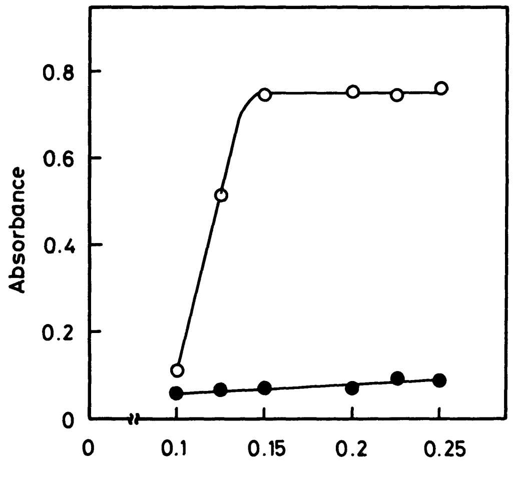

Fig. 3. Effect of concentration of ammonium molybdate on absorbance. [H_2SO_4]: 0.075 mol dm^{-3}, [Co-5-Cl-PADAP]: 25 μmol dm^{-3}. ○: 0.4 μg phosphorus against reagent blank, ●: reagent blank against water.

Table 1. Effects of Diverse Ions on the Determination of Phosphate[a]

Ion	Added as	Amount/μg	Relative error/%
As(V)	Na_2HAsO_4	0.4	+42
As(III)	As_2O_3	40	0
DS[b]	Na salt	4.0	+5
		40	+29
Si(IV)	Na_2SiO_3	400	+8
Fe(III)	$FeCl_3$	400	−2
ClO_4^-	$KClO_4$	400	+8
SCN^-	KSCN	400	+7
$S_2O_3^{2-}$	$Na_2S_2O_3$	400	+1

a) Phosphorus taken; 0.4 μg. b) Dodecyl sulfate.

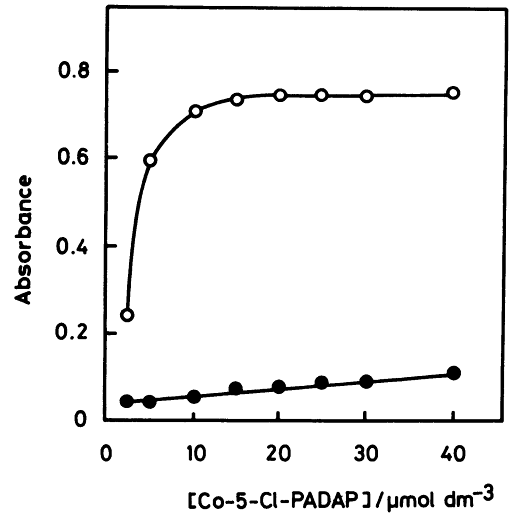

Fig. 4. Effect of concentration of Co-5-Cl-PADAP on absorbance. [H_2SO_4]: 0.075 mol dm^{-3}, [$(NH_4)_6Mo_7O_{24}$]: 0.225 mmol dm^{-3}. ○: 0.4 μg phosphorus against reagent blank, ●: reagent blank against water.

above the concentrations of 15 μmol dm^{-3}, while the absorbance of the reagent blank increased with the increase in the concentration of Co-5-Cl-PADAP. This increase in the absorbance of the reagent blank was explained by the formation and collection of the ion pair of molybdate with Co-5-Cl-PADAP. A concentration of 25 μmol dm^{-3} was chosen as the concentration of Co-5-Cl-PADAP.

Selection of Membrane Filter. The membrane filter collecting the ion pair of Co-5-Cl-PADAP with molybdophosphate must be dissolved into such organic solvents as DMF. Membrane filters made of cellulose nitrate, cellulose acetate, and a mixture of cellulose acetate and cellulose nitrate can be dissolved in DMF; a cellulose nitrate filter was used.

Effect of Pore Size of Membrane Filter. The effect of the pore size of the membrane filter on the absorbance was also studied. The constant absorbance of the ion pair was obtained in the pore-size range from 1.0 μm to 5.0 μm. The absorbance of the blank was also constant in this region. With the decrease in the pore size to less than 1.0 μm, the absorbance of the blank increased and the absorbance of the ion pair decreased. These results are similar to those reported in the collection of the ion pair of Malachite Green with molybdophosphate on a membrane filter.[7]

As the pore size of the membrane filter, 3.0 μm was used because of the constant absorbance of the ion pair and the facility of the filtration.

Other Conditions. The effect of the standing time on the absorbance was examined for the reaction after the addition of Co-5-Cl-PADAP. From 30 s to 5 min, the constant absorbance was obtained for both the ion pair and the reagent blank.

Two portions of 10 cm^3 of water were sufficient for washing the membrane filter with the ion pair.

As the solvent dissolving the membrane filter with the ion pair, DMF was used. Dimethyl sulfoxide and Methyl Cellosolve could also be used. Although 5.0 cm^3 of DMF was used for dissolving the membrane filter with the ion pair, 2.0 cm^3 was sufficient for a complete dissolution.

Calibration Curve, Sensitivity, and Precision. The calibration curve of phosphate was linear up to 0.4 μg of phosphorus, with a correlation coefficient of 0.999. The apparent molar absorption coefficient of phosphate was 2.9×10^5 dm^3 mol^{-1} cm^{-1}. The relative standard deviation was 1.7% for 0.4 μg of phosphorus.

Effect of Diverse Ions. The effect of diverse ions on the determination of phosphate is shown in Table 1. Arsenic(V) showed a serious positive error when it coexisted in the same amount as phosphorus. This is due to the formation and collection of the ion pair of molybdoarsenate with Co-5-Cl-PADAP. For the elimination of the error caused by arsenic(V), the reduction of arsenic(V) to arsenic(III) with thiosulfate[15] can be used. In the proposed method, arsenic(III) and thiosulfate showed no interference with the determination of phosphate.

Dodecyl sulfate, an anionic surfactant, exhibited a positive error of 5% when coexisting in an amount ten times that of phosphorus.

Although silicon showed a little positive error when coexisting in a 1000-fold amount, no serious problem arise in the determination of phosphate in natural waters.

Table 2. Determination of Phosphate–Phosphorus in Natural Waters[a]

Sample	Phosphorus content[b]/μg l^{-1}	
	Proposed method	Other method[c]
River water	3.6±0.3	4±1
Ground water	19.4±0.3	18±1

a) Sample taken; 5.0 cm^3. b) Mean±standard deviation (n=3). c) Flotation-spectrophotometry using Co-5-Cl-PADAP previously proposed by the present authors.[9]

Table 3. Spike and Recovery Test for Phosphate–Phosphorus in River Water[a]

Phosphorus/μg		Recovery/%
Added	Found	
0.000	0.035	—
0.050	0.089	108
0.100	0.130	95
0.150	0.193	105

a) Sample taken; 5.0 cm^3.

Iron(III) showed no interference with the proposed method. In the method using Malachite Green proposed by Matsubara et al.,[7] the presence of iron(III) in an amount equal to that of phosphorus caused an increase in the absorbance. Therefore, the pretreatment of samples with cation-exchange resin was required to remove iron(III). The proposed method is superior in the points of little interference caused by silicon and no interference by iron(III).

No ions commonly existing in natural waters, such as sodium, potassium, magnesium, calcium, chloride, and sulfate, interfere with the determination of phosphate. Organic substances, such as tartrate, citrate, ascorbate, and ethylenediaminetetraacetate, also showed no interference.

Determination of Phosphate in Natural Waters. The proposed method was applied to the determination of phosphate–phosphorus in natural waters. The samples were filtered with a 0.45 μm pore sized membrane filter and analyzed immediately or stored in a freezer until determination.[13] The results are shown in Table 2, in comparison with those of flotation-spectrophotometry using Co-5-Cl-PADAP previously proposed by present authors.[9] A satisfactory agreement was obtained between the two methods.

The spike and recovery test was examined for phosphate–phosphorus in another river water sample. The result, shown in Table 3, was satisfactory.

Enrichment of the Ion Pair on the Membrane Filter. Although the proposed method has a high sensitivity for the determination of traces of phosphate in natural waters, the enrichment of the ion pair on a membrane filter leads to an even more sensitive determination method.

To a 100-cm^3 portion of a sample, 1-cm^3 portions each of 7.5 mol dm^{-3} sulfuric acid, 22.5 mmol dm^{-3} ammonium molybdate, and 2.5 mmol dm^{-3} Co-5-Cl-PADAP were added. After filtration, the membrane filter with the ion pair was washed with three 30 cm^3 portions of water.

The calibration curve of phosphate was also linear up to 0.4 μg of phosphorus, with a correlation coefficient of 0.998. The molar absorption coefficient had the same value as that obtained by using 7 cm^3 of the sample. The absorbance of the reagent blank was 0.25, while that of 1 μg dm^{-3} of phosphrus was 0.19 against the reagent blank. The determination of 0.1 μg dm^{-3} levels of phosphorus can be made by using 100 cm^3 of the sample.

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