Notes

Simple and Rapid Spectrophotometric Determination of Trace Phosphate with Preconcentration of Phosphomolybdenum Blue Anion on Protonated Chitin

Suwaru Hoshi, Sachiko Kanagami, Masayuki Uto and Mutsuya Matsubara

Department of Environmental Engineering, Kitami Institute of Technology, Kitami 090, Japan

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The determination of inorganic phosphate samples, especially water samples, is very important. Most methods for determining phosphate are based on a spectrophotometric determination as its yellow phosphomolybdic acid and blue reduction products.[1] However, the sensitivity of those methods is not sufficient for the determination of low concentrations of phosphate in water samples. Therefore, the spectrophotometric methods of phosphate following various preconcentration techniques have recently been developed. Their methods are based on solvent extraction[2], flotation[3] and the collection on various supports including an ion-exchange resin[4], C_{18}-glass beads[5], Sephadex G-25[6] and a membrane filter.[7-9]

We have already proposed adsorption-elution and spectrophotometric methods for trace analysis by using a natural polymer, "chitin"[10,11], in which iron and copper as their 1,10-phenanthroline and neocuproine complexes were rapidly collected on chitin in the presence of suitable counter anions, and readily eluted with a small volume of an acetone–1 M (=mol/dm^3) acetic acid mixture. Up to a 100-fold concentration can be achieved with this method.

On the other hand, it is known that acetylamino groups of chitin are protonated in an acidic medium, acting as an anion exchanger (Fig. 1). This property of chitin was applied to the determination of a 10^{-8} mol level of nickel, copper and cadmium with flame atomic absorption spectrometry after adsorption-elution as their maleonitriledithiol anionic complexes.[12]

In this paper we report on the spectrophotometric determination of phosphate following the preconcentration procedures as follows: 1) the collection as its heteropolyblue anion on chitin and 2) the elution with a small volume of a mixture of organic solvent and 0.1 M ammonia buffer solution by using the above-mentioned property of chitin.

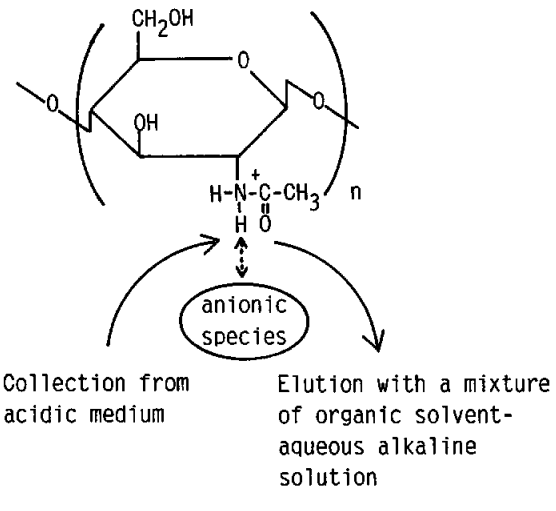

Fig. 1 Collection and elution scheme on protonated chitin.

Experimental

Reagents and apparatus

A 0.2 g sample of chitin powder (Nakarai Tesque) was packed into a polyethylene column (9 mm i.d.×70 mm height); the column was then washed successively with 10 cm^3 of 1 M hydrochloric acid and 20 cm^3 of distilled water before use.

A standard phosphate solution (0.1 mg/cm^3) was prepared by dissolving 0.2198 g of potassium dihydrogenphosphate into 500 cm^3 of water; it was further diluted as required.

A mixed reagent was prepared as described by Murphy and Riley:[13] a) 6 g of ammonium paramolybdate tetrahydrate and 0.24 g of potassium antimonyl tartrate were dissolved in 120 cm^3 of 12 M sulfuric acid and diluted to volume in a 500 cm^3 volumetric flask; b) 7.2 (w/v) L-ascorbic acid was then prepared. The mixed reagent was prepared by mixing a) : b)=5 : 1 when used. Other chemicals used were of analytical grade.

All absorbance measurements were made with a

Hitachi 200-100 spectrophotometer.

Standard procedure

Take a 100 cm³ sample solution containing up to 3 μg of phosphate; add 5 cm³ of the mixed reagent and stand for 10 min. Then pass the solution through the chitin column. After washing the column with 5 cm³ of water, elute the phosphomolybdenum blue (P-Mo blue) complex from chitin with 5 cm³ of a mixture of acetone–0.1 M ammonia buffer (pH 10) solution (7 : 3 v/v). After adding one drop of 10(w/v)% L-ascorbic acid solution to the eluate (to prevent aerial oxidation of the P-Mo blue complex), measure the absorbance of the eluent at 704 nm.

Analysis of river water

To 100 cm³ of river water which was filtrated with 1 μm pore sized glass fiber filter paper (Toyo Adovantec), add a 10 cm³ of 5 M sulfuric acid into a beaker and then heat the solution at 90 °C in a water bath for about 1 h. Cool and adjust to a pH of around 3 with concentrated ammonia. The phosphate contained in this sample was determined by the standard procedure.

Results and Discussion

Optimum condition for color development

The optimum condition for the color development of a P-Mo blue complex was studied in an aqueous solution. A mixed reagent was used because of simplicity and low reactivity to silica. The effect of the acid concentration was examined by using sulfuric acid. A maximum, constant absorbance was obtained below 0.1 M of the sulfuric acid concentration. A color development of the P-Mo blue complex was prevented over 0.1 M of the sulfuric acid concentration. A sample solution with a high concentration of acid must be adjusted to around pH 3 with concentrated ammonia. The effect of the reagent concentration was also examined. A maximum, constant absorbance was obtained by adding more than 2 cm³ of the mixed reagent to a 100 cm³ of solution containing 60 μg of phosphate. In this study, 5 cm³ of the mixed-reagent solution was added. After adding the reagent, a maximum, constant absorbance was obtained by standing for over 5 min.

Conditions for collection and elution

The elution of the P-Mo blue complex retained on a column of chitin was examined by using acetone, dimethylsulfoxide (DMSO), methanol, 0.1 M acetic acid and a 0.1 M ammonia buffer solution (pH 10). As shown in Fig. 2, the P-Mo blue complex was slightly eluted with these solvents alone and a mixture of organic solvents and a 0.1 M acetic acid solution. On the other hand, the elution of the P-Mo blue complex from chitin was easily achieved by using a 0.1 M ammonia buffer solution (pH 10) in 50 – 80(v/v)% of acetone or DMSO. These facts suggest that the P-Mo blue complex is largely

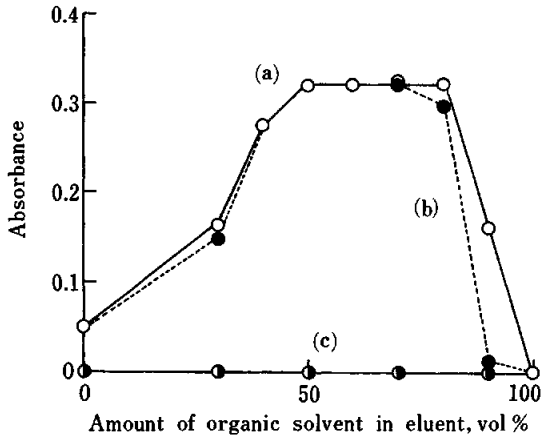

Fig. 2 Elution of the phosphomolybdenum blue complex from chitin with 5 cm³ of eluents. (a) acetone–0.1 M ammonia buffer (pH 10) (at 704 nm). (b) dimethylsulfoxide–0.1 M ammonia buffer (pH 10) (at 708 nm). (c) acetone–, dimethylsulfoxide– and methanol–0.1 M acetic acid. Collection on chitin was carried out from a 100 cm³ solution containing 3 μg of phosphate.

collected by an electrostatic interaction between the heteropolyblue anion and the surface of the protonated chitin. However, it is likely that the hydrophobic interaction also concerned the collection of the P-Mo blue complex, since elution from chitin affected the amount of organic solvents in the eluent. In this study a mixture of an acetone–0.1 M ammonia buffer (pH 10) solution (7 : 3, v/v) was used. The absorbance of the eluent decreased by about 5% within 1 h, due to an aerial oxidation of the heteropolyblue species. The absorbance could be made constant for 2 h by adding one drop of 10(w/v)% L-ascorbic acid to the eluent after elution.

The effect of the flow rate on the collection and elution was examined. The flow rate of sample solutions on the collection was varied from 5 to 80 cm³/min. The column was aspirated when the flow rate was more than 10 cm³/min. The P-Mo blue complex was sufficiently collected, even when the flow rate was 80 cm³/min. The P-Mo Blue complex retained on the chitin was readily eluted with 5 cm³ of the eluent within 1 min.

Collection from a 100 cm³ solution containing 3 μg of phosphate on a column with various amounts (0.05 – 0.3 g) of chitin was examined. The P-Mo blue complex was quantitatively collected on a column containing more than 0.1 g of chitin.

The collection of phosphate (3 μg) from various volumes (100 – 1000 cm³) was found to be constant over this range of sample volumes.

Five successive collections and elutions with 3 μg of phosphate on the same chitin, by repeating 1 M hydrochloric acid–water washing cycles, gave almost identical results.

Overall capacity

The overall capacity of a column packed with 0.2 g of

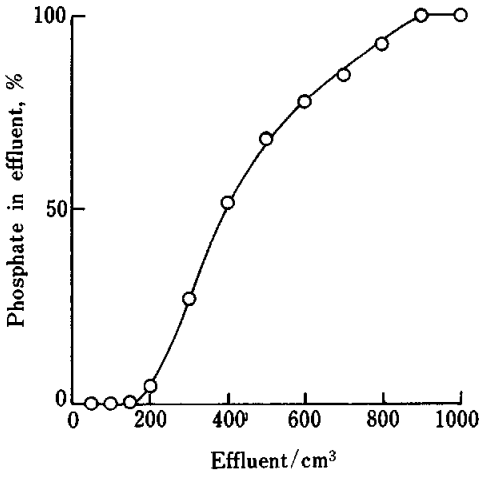

Fig. 3 Overall capacity curve: a solution containing 2 μg/cm³ of phosphate as its molybdenum blue complex was passed through a column of chitin (0.2 g) at a flow rate of 10 cm³/min.

chitin was determined by passing a solution containing 2 μg/cm³ of phosphate, as its heteropoly blue complex, at a flow rate of 10 cm³/min. The concentration of the remaining P-Mo blue complex in effluent was determined spectrophotometrically. The capacity was calculated from the difference in P-Mo blue complex concentration. The results are represented in Fig. 3. The overall capacity, as calculated from this curve, is 119.5 μmol/g of phosphate. The capacity is sufficient to collect a 0.1 μmol level of phosphate (equal to 3 μg), though its capacity is less than that of common anion-exchange resins.

Calibration and precision

The calibration curve obtained by the standard procedure was linear over a concentration range of 0.5 – 3 μg of phosphate in 5 cm³ of eluent. The relative standard deviation was 1.0% for 1.5 μg of phosphate (7 measurements).

Interference

Table 1 shows the effect of diverse ions. The tolerance limit was taken as the amount causing an error of ±3% in the absorbance. For a determination of 1.5 μg of phosphate, almost all of the substances which are commonly present in natural water do not interfere in concentrations of up to 1000 – 4000 times that of phosphate. Arsenic(III) can be tolerated in concentrations of up to 100 times. The presence of arsenic(V) in an amount equal to that of phosphate caused a positive error due to the formation of its heteropolyblue species with molybdate. The interference of arsenic(V) was eliminated by reducing to arsenic(III). Arsenic(V) on the determination of phosphate could be tolerated in concentrations of up to 50 times in the presence of thiosulfate (6000 μg) as a reducing agent.

Table 1 Effect of diverse ions

Ion	Added as	Amount added/μg	Recovery, %[b]
As(III)	As_2O_3	150	100.0
		750	106.3
As(V)	KH_2AsO_4	1.5	124.2
As(V)[a]		75	101.2
		150	105.6

P taken, 1.5 μg.

a. In the presence of 6000 μg of thiosulfate.

b. The value obtained in the phosphate solution without co-existent species is referred as 100%.

1500 – 6000 μg of Ca(II), Mg(II), Al(III), V(V), Cr(VI), Mn(II), Fe(III), Cu(II), Zn(II), NH_4^+, SiO_2, Cl^-, F^-, NO_3^-, ClO_4^-, I^-, NO_2^-, CO_3^{2-}, $S_2O_3^{2-}$ and dodecyl sulfate did not interfere.

Table 2 Determination of phosphate in river water[a]

P added/μg	P found/μg	Recovery of P added	
		μg	%
Muka river (A)			
none	1.92	—	—
0.5	2.43	0.51	102.0
1.0	2.93	1.01	101.0
1.5	3.41	1.49	99.3
2.0	3.96	2.04	102.0
Muka river (B)			
none	1.56	—	—
0.5	2.06	0.50	100.0
1.0	2.54	0.98	98.0
1.5	3.03	1.47	98.0
2.0	3.61	2.05	102.5
Tokoro river			
none	1.32	—	—
0.5	1.83	0.51	102.0
1.0	2.30	0.98	98.0
1.5	2.84	1.52	101.3
2.0	3.33	2.01	100.5

a. Sample volume of river water, 100 cm³.

Application

The proposed method was applied to the determination of phosphate in river-water samples. Phosphorus occurs in natural water as orthophosphate, condensed phosphates and organically bound phosphorus. The hydrolysis for condensed phosphates and organically bound phosphorus to orthophosphates has been examined by Motomizu *et al.*[2], and the hydrolysis method with sulfuric acid has been recommended. This method was used for the pretreatment of river water samples in this study; the effect of the acid concentration on the determination of phosphate was avoided by controlling the pH with concentrated ammonia (see Experimental section). Table 2 shows the results of an

analysis of original samples and samples to which known amounts of phosphate were added. The recovery of the added phosphate was nearly quantitative, as is shown in Table 2.

The present method is inferior regarding sensitivity, compared with the spectrophotometric methods for phosphate based on the formation of an ion-pair of the molybdophosphate with cationic-colored species.[2,3,9] However, the present method has advantages in that the procedure is relatively simple, silica and anion such as anionic surfactants and perchlorate can be torelated in higher concentrations, the collection and elution steps are rapid, and repeated use of the same chitin is possible. The proposed method may be applicable to the preconcentration of various colored anionic species used for spectrophotometry.

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