Determination of Phosphorus Based on the Formation of a Reduced Keggin-Type 12-Molybdophosphate Complex in an Aqueous-Organic Solution

Tadaharu UEDA,[†] Masashi HOJO, and Kouhei SHIMIZU

Department of Material Science, Faculty of Science, Kochi University, Kochi 780-8520, Japan

A very simple, easy and sensitive spectrophotometric manual determination method of phosphorus for the PO_4^{3-} ion, based on the formation of the reduced 12-molybdophosphate complex, was developed. The effect of the kind of water-miscible organic solvent and the concentration of organic solvent, ascorbic acid and HCl on the formation of the complex was investigated. The optimum determination condition was confirmed based on these results. In an aqueous- CH_3CN solution, a P-PO_4^{3-} of 0.01 – 20 mg l^{-1} could thus be determined. It was noted that the determination range of this study was wider than that of the phosphoantimonymolybdenum blue method (0.032 – 1 mg l^{-1}). The effect of foreign ions on the absorbance was examined. The P-PO_4^{3-} in river water and seawater sampled in Kochi was determined by this method. The results were also compared with those of the phosphoantimonymolybdenum blue method and capillary electrophoresis with an indirect detection method using K_2CrO_4 .

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Introduction

Extensive studies have been devoted to the determination of phosphorus as phosphate ion by the molybdenum blue method. There have been many reports on various modifications of this method in order to make the sensitivity and stability high. Various reductants, such as 1-amino-2-naphthol-4-sulfonic acid, stannous chloride(II), and ascorbic acid have been used to reduce the molybdophosphate complex.^{1–11} However, it is known to be difficult for methods using 1-amino-2-naphthol-4-sulfonic acid and stannous chloride(II) to control both the pH and the temperature.¹² It is also well known that solvent extraction with ethyl acetate, 1-butylalcohol, 2-methyl-1-propylalcohol, and so on, makes the sensitivity high.^{5,13–21} Motomizu *et al.* reported that the formation of colored ion-associate between molybdophosphate and Malachite Green could be utilized to determine phosphorus in the ultra-trace range from 0.018 to 1.0 ng cm^{-3} .²² These highly sensitive methods contain a few careful procedures and require skill to determine P-PO_4^{3-} with both high accuracy and precision. Molybdenum blue methods containing other metal ions, such as Sb(III), so-called phosphoantimonymolybdenum blue method, has also usually been used as manual batch procedures.^{2,3,23} Blomqvist and Westin reported in detail the effect of Cr(VI), Ge(VI), W(VI), and V(V) ions that have easily reacted with heteropolyoxometalate on the absorbance measurement in the phosphoantimonymolybdenum blue method.²⁴ Various flow-injection analysis methods, that analyze phosphorus automatically, have also been developed.^{2,3,25} It was also reported that organic phosphorus and hydrolyzable phosphorus were converted into the orthophosphate ion for determining

P-PO_4^{3-} .^{3,4,25–27}

In a previous report, the formation conditions of molybdophosphate and -arsenate complexes in aqueous solution were confirmed.²⁸ A few kinds of molybdophosphate complexes have been formed under high acidic conditions. The addition of water-miscible organic solvents, such as acetonitrile and acetone, made only 12-molybdophosphate stable. Maeda *et al.* examined the electrochemistry of 12-molybdophosphate in aqueous-organic solution.²⁹ The reduced form of 12-molybdophosphate was found to be more stable in aqueous-organic solution than in aqueous solution. Based on these results, optimizing the formation conditions of the reduced 12-molybdophosphate complex in aqueous-organic solution seems to enable one to determine a wider range of concentrations of P-PO_4^{3-} than the phosphoantimonymolybdenum blue method as a manual batch procedure.

Experimental

The absorption spectra were recorded on a Hitachi Model U-2000 spectrophotometer using silica cells with path lengths of 0.1 and 1 cm. Spectroscopic measurements under various conditions were made at $25 \pm 0.1^\circ\text{C}$ without any baseline correction; those under the optimum determining conditions were made at room temperature. All of the reagents were of analytical grade, and were used as received.

Results and Discussion

Optimization of the formation conditions of the reduced 12-molybdophosphate complex

Figure 1(a) shows the absorption spectrum for the 50 mM ($\text{M} = \text{mol dm}^{-3}$) Mo(VI) –1.0 M HCl–50% (v/v) CH_3CN –100 mM

[†] To whom correspondence should be addressed.
E-mail: chuji@cc.kochi-u.ac.jp

Fig. 1 Absorption spectra of reduced molybdophosphate complexes in aqueous-organic solution (a) and in aqueous solution (b), and the reduced phosphoantimonymolybdenum complex in aqueous solution (c).

Fig. 2 Changes in the absorbance at 790 nm as a function of the reaction time at 25°C for a 50 mM Mo(VI)-1.0 M HCl-1 mg l⁻¹ P-PO₄³⁻-50% (v/v) organic solvent-100 mM ascorbic acid system. The organic solvents were (a) acetonitrile, (b) acetone, (c) THF, (d) methanol, and (e) 1,4-dioxane.

ascorbic acid system containing 1 mg l⁻¹ P-PO₄³⁻. The absorption maximum appeared at 790 nm. For a comparison, Figs. 1(b) and (c) show the absorption spectra for the 50 mM Mo(VI)-1.0 M HCl-100 mM ascorbic acid system and the Mo(VI)-Sb(III)-H₂SO₄-ascorbic acid system (referred to the condition of Ref. 3), respectively, both containing 1 mg l⁻¹ P-PO₄³⁻. The absorption maxima appeared at 820 and 890 nm, respectively. Figures 1(a) and (b) indicate the formation of the reduced form of the 12-molybdophosphate anion, and Fig. 1(c) the formation of the reduced form of PSb₂Mo₁₀O₄₀.³⁰ Harwood *et al.* demonstrated that the absorption spectrum for the Mo(VI)-Sb(III)-H₂SO₄-ascorbic acid system containing an excess of phosphate ion was identical to Fig. 1(b).³¹ This result indicated that the determination of phosphorus in a solution containing >1 mg l⁻¹ P-PO₄³⁻ was difficult, because the reduced forms of the PSb₂Mo₁₀O₄₀ complex were converted into the reduced forms of the PMo₁₂O₄₀ complex. The absorbance at 790 nm was monitored, as discussed below, in solutions under

Fig. 3 Changes in the absorbance at 790 nm as a function of the reaction time at 25°C for a 50 mM Mo(VI)-1.0 M HCl-1 mg l⁻¹ P-PO₄³⁻-100 mM ascorbic acid system containing various amounts of acetonitrile. [CH₃CN]/%(v/v) = (a) 0; (b) 30; (c) 40; (d) 50; (e) 60.

Fig. 4 Changes in the absorbance at 790 nm as a function of the reaction time at 25°C for a 50 mM Mo(VI)-1 mg l⁻¹ P-PO₄³⁻-50% (v/v) CH₃CN system containing various concentration of ascorbic acid. [Ascorbic acid]/mM = (a) 10; (b) 25; (c) 50; (d) 100.

various conditions to optimize the formation condition of reduced 12-molybdophosphate anion. In order to choose the best organic solvent in which reduced 12-molybdophosphate anions would form stably, the absorbance was measured as a function of the reaction time for 50 mM Mo(VI)-1.0 M HCl-1 mg l⁻¹ P-PO₄³⁻-100 mM ascorbic acid containing various kinds of 50% (v/v) organic solvents, such as acetonitrile, acetone, tetrahydrofuran, methanol, and 1,4-dioxane (Fig. 2). For a comparison, in aqueous solution, the measurement was also made as shown in Fig. 3(a). The absorbance kept increasing for at least 2 h, except for that in 50% (v/v) acetonitrile.

Figure 3 shows the change in the absorbance at 790 nm as a function of the reaction time for a 50 mM Mo(VI)-1.0 M HCl-1 mg l⁻¹ P-PO₄³⁻-100 mM ascorbic acid system containing various amounts of CH₃CN. The absorbance was stable at C_{CH₃CN} > 50% (v/v). However, if C_{CH₃CN} > 50% (v/v) was added, the amount of the test solution became very small. Therefore, the amount of CH₃CN in the mixed solution must be 50% (v/v).

In order to choose the best reductant, various reductants, such

Fig. 5 Absorbance at 790 nm as a function of the HCl concentration for a 50 mM Mo(VI)-1 mg l⁻¹ P-PO₄³⁻-100 mM ascorbic acid-50% (v/v) CH₃CN system. The absorbance was measured after standing at room temperature for 1 h. Path length, 1 cm.

as stannous chloride(II), hydrazine and ascorbic acid, were examined; but it was difficult to control the temperature and concentration of the acid and reductants, except for ascorbic acid. Figure 4 shows the change in the absorbance at 790 nm as a function of the reaction time for a 50 mM Mo(VI)-1.0 M HCl-1 mg l⁻¹ P-PO₄³⁻-50% (v/v) CH₃CN system containing various concentrations of ascorbic acid. When more than 25 mM of ascorbic acid was added, the absorbance ultimately became the same value. With increasing concentration of ascorbic acid, the absorbance quickly became unchangeable. At [ascorbic acid] = 100 mM, it required *ca.* 30 min to achieve a constant value of the absorbance. When the solution was heated, the color of the solution turned blue and the absorbance quickly became constant in spite of the low concentration of ascorbic acid. However, heating the solution was excluded from the set of determination procedures for simplicity.

In order to obtain the limiting conditions of the reduced 12-molybdophosphate complex, the absorbance was measured for a series of 50 mM Mo(VI)-1 mg l⁻¹ P-PO₄³⁻-100 mM ascorbic acid-50% (v/v) CH₃CN systems containing various concentrations of HCl (0.7 – 2.0 M). Even in the absence of phosphate ions, the solution gave a blue color due to the reduced isopolymolybdates at [HCl] < 1.0 M. Therefore, the increment of the absorbance at [HCl] < 1.0 M (Fig. 5), was attributed to the formation of reduced isopolymolybdates and reduced 12-molybdophosphate. It was found that the reduced 12-molybdophosphate anion was obtained at up to 1.9 M of HCl concentration. At [HCl] = 1.0 – 1.3 M, the absorbance was constant. These results indicated that the optimum condition for the determination was 50 mM Mo(VI)-1.0 – 1.3 M HCl-100 mM ascorbic acid-50% (v/v) CH₃CN. Finally, the acid concentration was chosen to be 1.2 M, while taking into account of interference from the Si(IV) ion (see below) and the preparation of the stock solution.

Recommended procedure

A stock solution of 1 M ascorbic acid was prepared for every measurement. A 1.0984 g quantity of KH₂PO₄³⁻, which had been dried for 2 h in an oven at 110°C, was dissolved in 250 ml of distilled water (1000 mg l⁻¹ P-PO₄³⁻). An aqueous solution containing 12.0975 g of Na₂MoO₄·2H₂O and 104 ml of conc.

Fig. 6 Calibration curve of P-PO₄³⁻ for a 50 mM Mo(VI)-1.2 M HCl-100 mM ascorbic acid-50% (v/v) CH₃CN system. The absorbance at 790 nm was measured after standing at room temperature for 1 h and is shown as the value for a 1 cm path length.

Table 1 Effects of foreign ions on the determination of P-PO₄³⁻

	Concentration/ mg l ⁻¹	Absorbance	Relative error, %
None		0.687	
Na ⁺	1000	0.689	+0.3
K ⁺	1000	0.689	+0.3
Mg ²⁺	1000	0.702	+2.2
Ca ²⁺	1000	0.700	+1.9
V(V)	10	0.694	+1.0
	1	0.687	0
Fe(II)	10	0.706	+2.6
N-NO ₃ ⁻	10	0.685	-0.3
	1	0.688	+0.1
N-NO ₂ ⁻	10	0.662	-3.6
	1	0.686	-0.1
S-SO ₄ ²⁻	10	0.686	-0.1
	1	0.683	-0.6
S-SO ₃ ²⁻	10	0.689	+0.3
	1	0.689	+0.3
Si(IV)	10	0.692	+0.7
	1	0.690	+0.4
As-AsO ₄ ³⁻	1	1.028	+49.6
P-PHO ₃ ²⁻	1	0.713	+3.8
P-P ₂ O ₇ ⁴⁻	1	1.079	+57.1

[P-PO₄³⁻] = 1 mg l⁻¹. 50 mM Mo(VI)-1.2 M HCl-100 mM ascorbic acid-50% (v/v) CH₃CN. Measured after standing at room temperature for 1 h. Path length, 1 cm.

HCl was placed in a 250 ml volumetric flask, which was diluted to the mark with distilled water. This solution was stable in a polyethylene bottle for at least 2 months. A 5 ml aliquot of the Mo(VI)-HCl solution and 10 ml of CH₃CN were placed in a 20 ml volumetric flask. To the mixture were added an appropriate volume of the sample solution and 2 ml of the ascorbic acid stock solution; the solution was then diluted to the mark with distilled water and left standing for 1 h at room temperature. The absorbance at 790 nm was measured, while referring to the blank solution. The determination range of P-PO₄³⁻ in an aqueous-CH₃CN solution was from 0.01 to 20 mg l⁻¹, as shown in Fig. 6. This is wider than that of the phosphoantimonymolybdenum blue method, of which the

Table 2 Phosphorus, pH and conductivity in river water and seawater samples

Sampling point	pH	Conductivity/ μS	Method A ^a / mg l^{-1}	Method B ^b / mg l^{-1}	Method C ^c / mg l^{-1}
Kagami river	7.9	79	<0.032	ND ^d	<0.01
Yoshino river	7.2	161	0.14	ND	0.15
Enoguchi river	7.1	6200	0.22	0.21	0.22
Kochi harbor	7.2	8100	0.08	ND	0.08

a. Phosphoantimonylmolybdenum blue method. b. Capillary electrophoresis using indirect method. c. This study. d. Not detected.

determination range has been reported to be $0.032 - 1 \text{ mg l}^{-1}$.³² The concentration of P-PO_4^{3-} was determined by the corresponding calibration curve.

Interference from foreign ions

The effects of foreign ions on the determination of phosphorus are listed in Table 1. Since the excess amounts of alkaline and alkaline earth metals in a sample solution have not affected the formation of reduced 12-molybdophosphate, this method could be applied to the determination of phosphorus in seawater. A large amount of ascorbic acid had little effect on oxidants, such as nitrate and nitrite ions. It is known that the formation conditions of reduced molybdosilicates are less acidic than those of reduced molybdophosphates.³³ Under the optimum analytical conditions, the silicate ion had only a small effect on the determination. When the determination condition of the 50 mM Mo(VI) -1.0 M HCl -100 mM ascorbic acid-50% (v/v) CH_3CN system was applied, the silicate ion caused significant interference. Unfortunately, the arsenate ion interfered, since the reduced 12-molybdoarsenate as well as the reduced 12-molybdophosphate had formed in an aqueous- CH_3CN solution. Previously, heteropolymolybdates containing PHO_3^{2-} and $\text{P}_2\text{O}_7^{4-}$ were prepared and characterized in aqueous-acetonitrile and -acetone solutions.^{34,35} Based on these formation reactions, the direct spectrophotometric determination method of PHO_3^{2-} and $\text{P}_2\text{O}_7^{4-}$ ions in an aqueous-acetone solution was also developed.³⁶ In fact, PHO_3^{2-} and $\text{P}_2\text{O}_7^{4-}$ ions also brought about positive errors in an aqueous- CH_3CN solution. If we try to determine P-PO_4^{3-} in sample solutions containing an excess of AsO_4^{3-} , PHO_3^{2-} , and $\text{P}_2\text{O}_7^{4-}$ ions, they must be analyzed by other methods, such as atomic absorption spectroscopy, inductively coupled argon-plasma emission spectroscopy, and ion chromatography. However, since it has been well known that the concentrations of AsO_4^{3-} , PHO_3^{2-} , and $\text{P}_2\text{O}_7^{4-}$ ions in river and seawater are much less than that of the PO_4^{3-} ion, they should not interfere seriously with the determination.^{23,37,38}

Analysis of phosphorus in river and seawater, and comparison with the other methods

The present method was applied to the determination of phosphorus in water sampled from a few rivers in Kochi city and Kochi harbor. For a comparison, the same water samples were also analyzed by the phosphoantimonylmolybdenum blue method and by capillary electrophoresis with an indirect method (K_2CrO_4 reagent).³⁹ These results are listed in Table 2. The pH and conductivity of the sample waters were also measured. The results obtained by the method used in this study coincided well with those of other methods.

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