Sensitive Spectrophotometric Determination of Phosphate Using Silica-Gel Collectors

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Phosphate, 3 – 10 nmol, in 1 dm³ of natural-water samples was quantitatively collected along with 10 µmol of Fe(III) ion onto a silica-gel collector in the pH range of 5.4 – 6.2. The amount of Fe(III) ion needed was limited to such a low level that the Pyrocatechol Violet method could be applied without removing the Fe(III) ion, providing a superior determination method for phosphate. Surface-water samples of the north basin of Lake Biwa and the Tanabe Bay in the Wakayama prefecture were selected as being representative of natural water with extremely low phosphate concentrations, and were found to have phosphate concentrations of 3.68 and 4.31 nmol dm⁻³, respectively.

(Received June 30, 2003; Accepted November 13, 2003)

Introduction

Phosphate is an essential substance for the growth and reproduction of living organisms, and one of the main controlling factors for the characteristics of streams, rivers, lakes, and oceans. Improvements in the sensitivity and precision of phosphate analysis and simplification of the analytical procedure should significantly benefit various research fields, such as limnology, oceanography, and environmental chemistry. Currently, there are several standard methods that satisfy such demands. One of the most popular methods is the molybdenum blue reaction with coexisting antimony, proposed by Murphy and Riley,¹ which has been applied not only to seawater, but also to lake water and to evaluating the quality of water and wastewater.² This method is superior both in sensitivity and precision (detection limit of 0.3 µmol dm⁻³, relative standard deviation of 2% at 7 µmol dm⁻³), and the analysis can be automated with the flow-injection method.³

However, there is still a need to improve the method for determining phosphate in natural water by one or two orders in sensitivity, since the dissolved phosphate concentrations of most natural-water samples collected from the headstreams of rivers, the surface of lakes during summer stratification, or the surface of pelagic water are significantly below the detection limit of the conventional determination methods. For example, in the surface water of lakes and oceans, where natural purification, such as the removal of nutritious phosphate by phytoplanktons proceeds actively, phosphate concentrations are often decreased below 0.01 µmol dm⁻³. For such a low level of phosphate, a more sensitive method has been expected to substantiate limnologically and oceanographically the processes of natural purification.

In the past, many methods have been proposed for determination of phosphate in natural water. For example, Ishibashi and Tabushi⁴ proposed a method that combines the preconcentration of phosphate using hydrated iron oxides as adsorbents and the solvent extraction of phosphomolybdate to butyl acetate, and succeeded in determining phosphate in seawater at concentrations of 0.05 – 0.1 µmol dm⁻³. Hori *et al.*⁵ proposed an indirect determination method in which phosphomolybdate is first extracted in a mixed solvent of methyl isobutyl ketone and cyclohexane. Then, the molybdenum in the organic solvent is quantified by the Pyrocatechol Violet method. This analytical method improved the detection limit to 0.02 µmol dm⁻³. Motomizu *et al.* attempted to improve the sensitivity according to a different principle.^{6,7} In their method, the phosphomolybdate produced in 5 – 100 cm³ of sample water is made to form an ion-associate with cationic dyes, such as Ethyl Violet⁶ or Malachite Green,⁷ and then extracted in a solvent. They achieved a detection limit of 0.003 µmol dm⁻³ with this method.

In this study, we found that phosphate in a sample solution can be collected by silica gel in the presence of a small amount of Fe(III) ion. Under the most appropriate conditions, the phosphate can be quantitatively adsorbed onto silica gel at pH 5.4 – 6.2 with 10 µmol of Fe(III) ion. Thus, this method can be used to effectively preconcentrate phosphate in natural water, such as lake water and seawater. By combining this preconcentration method with the Pyrocatechol Violet method,⁵ it is possible to quantify phosphate within concentrations of 0.003 – 0.01 µmol dm⁻³.

Experimental

Reagents

A 11:9 (v/v) methyl isobutyl ketone/cyclohexane-mixed solvent, a 0.2% pyrocatechol sulfonphthalein (Pyrocatechol

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Violet) solution, and a 0.5% methyltriocetylammmonium chloride (Capriquat)-ethanol solution were prepared according to Hori *et al.*⁵

A 0.12 mol dm⁻³ sodium molybdate solution, a 0.1000 mol dm⁻³ potassium dihydrogen phosphate standard solution, and a 0.1 mol dm⁻³ ferric chloride solution were also used. Artificial seawater was prepared following a method of Lyman and Fleming.⁸

A dispersed silica gel solution was prepared in the following manner. A 7.10-g dose of Na₂SiO₃·9H₂O was dissolved in 40 cm³ of distilled water, to which 5 cm³ of conc. HCl was added and mixed thoroughly. The solution was continuously mixed while NaOH solution was added in drops to gradually increase the pH to 8. The viscosity of the solution gradually increased with the pH to attain a starch-like texture. This silica gel was separated from the solution by centrifugation, washed 5 times with distilled water, dispersed in distilled water and diluted to 50 cm³. For every phosphate sample solution, 1 cm³ of this dispersed silica gel solution was used.

Standard procedures

Phosphate in natural water is collected using silica gel in the presence of Fe(III) ion. Since the Fe(III) ion needed for collection is kept at a low level, it is possible to apply the Pyrocatechol Violet method,⁵ which enables the achievement of a superior determination method for phosphate. Below is an outline of the standard analytical procedures.

(i) Aliquots of 0.1 cm³ of 0.1 mmol dm⁻³ ferric chloride solution and 1 cm³ of the dispersed silica gel solution are added to 1 dm³ of a water sample previously filtered through a nucleopore filter with a pore size of 0.40 µm. The solution is mixed thoroughly for 1 h after adjusting the pH to 5.4–6.2 with a HCl or NaOH solution. Then, the precipitate, which consists of silica gel and phosphate adsorbed onto the gel with Fe(III) ion, is collected by filtration through the same filter as mentioned above. The phosphate in the precipitate on the filter is dissolved with 3.5 cm³ of heated 6 mol dm⁻³ HCl, and collected in a 50-cm³ centrifuge tube with a ground-in stopper. The filtrate and the wash liquids are combined and diluted with distilled water to ca. 35 cm³. This HCl solution, which contains phosphate, is analyzed in the following step: (ii) the sample solution obtained as mentioned above is mixed with 3.5 cm³ of a 0.12 mol dm⁻³ sodium molybdate solution. This solution, in which phosphomolybdate is formed, is mixed with 7 cm³ of a 11:9 (v/v) methyl isobutyl ketone/cyclohexane-mixed solvent and shaken vigorously for 5 min for extracting the phosphomolybdate. After separation of the organic and aqueous layers, 5 cm³ of the organic layer is transferred into a 10-cm³ centrifuge tube with a ground-in stopper. Then, 2.5 cm³ of 1 mol dm⁻³ NaOH is added to the organic solution, and the mixture is shaken for 1 min, followed by the addition of 1.5 cm³ of 2.5 mol dm⁻³ HCl, 1 cm³ of a 0.2% Pyrocatechol Violet solution, and 0.5 cm³ of a 0.5% Capriquat solution. The mixture is shaken again for 1 min. After separation of the organic and aqueous layers, the absorbance of the organic layer in 1-cm cell is measured at 545 nm.

Results

Calibration curve

After 35 cm³ portions of 0.6 mol dm⁻³ HCl were transferred into a 50-cm³ centrifuge tube with a ground-in stopper, 2.5, 5, 7.5, and 10 nmol portions of phosphate were added to them. Step (ii) of the standard procedures was applied to the solutions.

Fig. 1 (a) Adsorption curves of phosphate in the presence of Fe(III) ion. The adsorptions of 20 nmol of phosphate in 1 dm³ of water with (curve 1) and without (curve 2) the addition of silica gel were compared in the presence of Fe(III) ion. (b) Formation curves for the chemical species of phosphate and Fe(III) ions. The chemical species of phosphate ion (solid lines) [1, H₃PO₄; 2, H₂PO₄⁻; 3, HPO₄²⁻; 4, PO₄³⁻] and Fe(III) ion (dashed lines) [5, Fe³⁺; 6, FeOH²⁺; 7, Fe(OH)₂⁺; 8, Fe(OH)₃(aq); 9, Fe(OH)₄⁻] are given as a function of the pH. The equilibrium constants used in the calculations were, for phosphate ion, log K_{a1} = -2.2, log K_{a2} = -7.2, and log K_{a3} = -12.3, and for Fe(III) ion, log K_{a1} = -2.19, log K_{a2} = -3.48, log K_{a3} = -6.33, and log K_{a4} = -9.6 (Stumm and Morgan).⁹

The values of the absorbance measured against a reagent blank were 0.209 for a solution whose content of phosphate was 2.5 nmol, 0.428 for 5 nmol of phosphate, 0.632 for 7.5 nmol, and 0.836 for 10 nmol. The slope of the linear calibration curve drawn from these values was 0.0838 nmol⁻¹ and the correlation coefficient was 0.9999.

After distilled water, and 2.5 and 10 nmol dm⁻³ potassium dihydrogen phosphate solutions were prepared, steps (i) and (ii) of the standard procedures were applied to 1 dm³ portions of these solutions. The values of the absorbances measured against the same reagent blank as above were -0.013 for a sample solution containing no phosphate, 0.218 for the content of 2.5 nmol of phosphate, and 0.838 for 10 nmol. Comparing these results with those mentioned above for the same contents of phosphate, they were in good agreement (their differences were 0.009 for 2.5 nmol of phosphate, 0.002 for 10 nmol, and -0.013 for no phosphate). This shows that phosphate is quantitatively collected in step (i), and that the calibration curve can be drawn only by applying step (ii) without the preconcentration procedures of step (i).

Determination of phosphate in natural waters

Two samples were taken from the surface water of the north basin of Lake Biwa (depth 73 m, sampled in Dec. 1999) and the Tanabe Bay in Wakayama prefecture (depth 30 m, sampled in Aug. 1999), and analyzed twice (*n* = 2) according to the standard procedures. The concentrations of phosphate in the lake water were 3.67 and 3.68 nmol dm⁻³, and those in the seawater were 4.30 and 4.32 nmol dm⁻³.

Fig. 2 Adsorption curves of phosphate in seawater in the presence of Fe(III) ion. The adsorptions of 20 nmol of phosphate in 1 dm³ of artificial seawater with (curve 1) and without (curve 2) the addition of silica gel were compared in the presence of Fe(III) ion.

Discussion

The adsorption curves of phosphate

Curve 1 in Fig. 1a is the adsorption curve of phosphate in a 20 nmol dm⁻³ potassium dihydrogen phosphate solution onto silica gel in the presence of Fe(III) ion. This adsorption curve has two local maxima. The curve begins to rise at around pH 3 and reaches the first maximum with a constant value at pH 5.4–6.2. It then drops off to 80% of the peak value at pH 6.5, and then rises again above pH 6.5 to reach the second maximum at pH 8.

For a comparison, the adsorption curve of phosphate onto silica gel without Fe(III) ion was investigated. It was found that phosphate was scarcely adsorbed onto silica gel alone. Next, the adsorption of phosphate to iron hydroxide (curve 2) was studied. The rate of adsorption was around 20% in the pH range of 3–6.2, increased above pH 7, and reached a maximum at pH 8. This adsorption curve resembled curve 1 in the pH range of 7–10. This indicates that the adsorption of phosphate at pH 7–10 can be attributed to the adsorption solely onto iron hydroxide, but that the presence of both Fe(III) ion and silica gel is necessary for adsorption at pH 5.4–6.2.

The adsorption curve was obtained for artificial seawater in a similar manner (Fig. 2), and the phosphate could be collected effectively by adsorption onto silica gel in the presence of Fe(III) ion, even for seawater samples. Curve 1 in Fig. 2 indicates that phosphate in seawater is quantitatively adsorbed at pH 5.4–7.4. This pH range was wider than the above range for fresh water. For a comparison, curve 2 in Fig. 2 shows the adsorption curve of phosphate to iron hydroxide. Almost the same as that for fresh-water samples, the local maximum of the adsorption curve was reached at pH 8.

It is concluded that by using silica gel as collectors in the presence of Fe(III) ion, phosphate can be adsorbed quantitatively over a relatively wide pH range of 5.4–6.2 for both fresh-water and seawater samples.

The desorption curve of phosphate

Figure 3 shows the desorption curve of phosphate collected on silica gel in the presence of Fe(III) ion, along with the adsorption curve for a comparison. The adsorption and desorption of phosphate was irreversible for the pH, and stronger acidity was necessary for the desorption. However, as can be seen from the figure, phosphate is desorbed quantitatively into the aqueous solution below pH 1.

Fig. 3 Desorption curve of phosphate from silica gel in the presence of Fe(III) ion. Phosphate (2 μmol), which had been adsorbed onto silica gel in the presence of Fe(III) ion, was desorbed into the solution by varying the pH. For a comparison, the adsorption curve (dashed line) is superimposed.

Chemical form of the phosphate adsorbed

Although the adsorption mechanism for the three-component system of phosphate-Fe(III)-silica gel has been left unsolved, it is ensured unambiguously in the preceding paragraphs that phosphate ion, even at extremely low concentrations, is collected quantitatively in the presence of both Fe(III) ion and silica gel. The most probable mechanisms are: (1) chemical species of phosphate-Fe(III) formed in the aqueous phase are adsorbed on the silica gel, and (2) phosphate ions in the aqueous phase are adsorbed on the silica gel containing Fe(III) ion. In the present paper, we can say that mechanism (1) is anticipated from the similarity between the adsorption curves of phosphate ion (Fig. 1a) and the distribution curves of phosphate and Fe(III) ions (Fig. 1b).

Figure 1b shows the distribution of the chemical forms of phosphate and Fe(III) ions as functions of the pH. Among the possible combinations of the chemical forms of phosphate ion (H_3PO_4 , H_2PO_4^- , HPO_4^{2-} , PO_4^{3-}) and Fe(III) ion (Fe^{3+} , FeOH^{2+} , Fe(OH)_2^+ , $\text{Fe(OH)}_3(\text{aq})$, Fe(OH)_4^-), the combinations of the dominant species are (i) H_3PO_4 and Fe^{3+} at pH < 2.2 (curves 1 & 5), (ii) H_2PO_4^- and FeOH^{2+} (curves 2 & 6) at pH = 2.2–3.5, (iii) H_2PO_4^- and Fe(OH)_2^+ (curves 2 & 7) at pH = 3.5–6.3, (iv) H_2PO_4^- and $\text{Fe(OH)}_3(\text{aq})$ (curves 2 & 8) at pH = 6.3–7.2, (v) HPO_4^{2-} and $\text{Fe(OH)}_3(\text{aq})$ (curves 3 & 8) at pH = 7.2–9.6, and (vi) HPO_4^{2-} and Fe(OH)_4^- (curves 3 & 9) at pH > 9.6. As aforementioned, the adsorption of phosphate onto silica gel in the presence of Fe(III) ion occurs especially with an adsorption rate of over 50% in the pH range of 4–6.5 (curve 1 in Fig. 1a). The combination of the chemical forms of phosphate and Fe(III) ions in this range, which may interact together, is the combination (iii) of H_2PO_4^- and Fe(OH)_2^+ . The reaction product, therefore, is considered to be $\text{H}_2\text{PO}_4\text{-Fe(OH)}_2$ or FePO_4 . These are neutral species, and when phosphate and Fe(III) ions are present in high concentrations, the solubility product is easily exceeded and precipitation occurs. However, when the two are present only in extremely low concentrations and the solubility product is not exceeded, there is almost no precipitation present. When silica gel coexists as a third component in such a condition, FePO_4 is collected onto the gel. This is in good agreement with the observation of the remarkable phosphate adsorption in the pH range of 5.4–6.2 (curve 1 in Fig. 1a) in a mixed system of phosphate, Fe(III) ion, and silica gel.

The combinations of dominant species of phosphate and Fe(III) ions in the pH range of 6.5–9 are (iv) H_2PO_4^- and

Fig. 4 Effect of Fe(III) ion on the Pyrocatechol Violet method. The method was applied to sample solutions in the Fe(III) ion concentration range of 0 – 1.43 mmol dm⁻³ and a constant phosphate concentration (0.285 μmol dm⁻³). The absorbances at 545 nm were plotted against the Fe(III) ion concentrations.

Fe(OH)₃(aq) or (v) HPO₄²⁻ and Fe(OH)₃(aq). This implies that, in the absence of silica gel, the phosphate adsorbed onto iron hydroxide is either H₂PO₄⁻ or HPO₄²⁻.

The effect of Fe(III) ion on phosphate determination

The Pyrocatechol Violet method was applied to samples with a constant phosphate concentration and varying Fe(III) ion concentrations (Fig. 4). Positive errors were observed at a Fe(III) ion concentration ≥ 0.5 mmol dm⁻³. Because the Fe(III) ion concentration is limited to 0.3 mmol dm⁻³ in the sample solution obtained in step (i) of the standard procedures, the

Pyrocatechol Violet method can be performed with high sensitivity without any negative effects from the Fe(III) ion.

Acknowledgements

We are grateful to the faculty members of the Center for Ecological Research, Kyoto University, and of the Seto Marine Biological Laboratory, Kyoto University, for the use of their observation facilities on Lake Biwa and Tanabe Bay. This work was supported in part by Grants-in-Aid for Scientific Research (Category: C-15550069) from the Ministry of Education, Culture, Sports, Science and Technology.

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