Solvent Extraction-Spectrophotometric Determination of Trace Orthophosphate in Water by Stopped-Flow Time Difference Analysis Technique

Ken-ichiro KANAYA* and Keitaro HIROMI**

*Japan Food Research Laboratories, Osaka Branch, Toyotsu, Suita, Osaka 564

**Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606

For the determination of phosphate from $\mu\text{g l}^{-1}$ to sub- $\mu\text{g l}^{-1}$ level in water, the "stopped-flow time difference analysis" technique (a sort of end-point assay using the stopped-flow method) was used by combining the "Molybdenum Blue" method and the pre-concentrating solvent extraction of 12-molybdophosphate. Isobutyl alcohol and stannous chloride were used as the solvent and the reductant, respectively. The reducing reaction was fast and the colored complex formed by the reaction was unstable. Phosphate concentration was easily determined by analyzing the reaction curves recorded for only 0.5 s. The apparent molar absorptivity of phosphate was about $2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ at 730 nm, and the detection limit was $0.3 \mu\text{g l}^{-1}$. The calibration curve was linear from zero to $5 \mu\text{g l}^{-1}$. The method was applied to the determination of phosphate in tap water samples; the relative standard deviation was less than 3% and the recovery of $20 \mu\text{g l}^{-1}$ phosphate was $100 \pm 6\%$.

Keywords Stopped-flow method, water analysis, phosphate, Molybdenum Blue method, solvent extraction

The eutrophication problem in environmental water requires the methods which provide sensitivity high enough to determine phosphate from $\mu\text{g l}^{-1}$ (ppb) to sub- $\mu\text{g l}^{-1}$ (sub-ppb) levels.

The spectrophotometric methods for phosphate are usually based on the formation of 12-molybdophosphate (abbreviated to 12MPA). Since 12MPA is easily soluble in organic solvents, solvent extraction techniques are often used to concentrate 12MPA for enhancing the sensitivity and/or for removing interfering substances containing condensed phosphates.¹⁻³

If the solvent extraction is not used, the widely used "Molybdenum Blue" method is usually of low sensitivity (the molar absorptivity is usually about $4 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) compared with the "Malachite Green" method⁴⁻⁷ (the molar absorptivity is usually about $1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$), which is based on the colored complex formation of 12MPA with Malachite Green. Therefore, in the previous paper⁸, the authors have reported the "Malachite Green" method combined with the so-called "stopped-flow time difference analysis (abbreviated to SFTDA)" technique⁹, which is a sort of end-point assay technique, and is superior to the ordinary spectrophotometry in numerous characteristics.⁸⁻¹⁰ This rapid and sensitive spectrophotometric method without any solvent extraction was found to have several advantages over the ordinary "Malachite Green" method, but it was unsuitable to determine trace phosphate below $\mu\text{g l}^{-1}$

level, because the detection limit was $2 \mu\text{g l}^{-1}$.⁸ On the other hand, in spite of several trials in our laboratory, the suitable analytical conditions for the SFTDA technique combined with the solvent extraction and the "Malachite Green" method have not been obtained.

In this paper, the SFTDA technique combined with the solvent extraction and the "Molybdenum Blue" method is developed in order to provide higher sensitivity than that of the method previously reported.⁸

Experimental

Apparatus

A stopped-flow spectrophotometer (Union Giken SF-70) was used. The path length of the cell was 10 mm. The dead time of the flow-system was about 2 ms, as determined by the method of Nakatani and Hiromi.^{11,12}

Chemicals and reagents

All chemicals were of analytical reagent grade and were used without further purification. Deionized and distilled water was used.

Stock solutions. 1) 0.012 M ammonium molybdate in 3.2 M sulfuric acid: Dissolve 15 g of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in about 150 ml of water, add 800 ml of 4 M H_2SO_4 , and dilute to 1000 ml with water. 2) 0.35 M

sulfuric acid. 3) 1 M hydrochloric acid in 90% (v/v) ethanol.

Standard solution. Standard solution of orthophosphate was prepared with potassium dihydrogen phosphate, KH_2PO_4 .

Color-developing (reducing) reagent. Dissolve 0.4 g of stannous chloride, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, in 100 ml of isobutyl alcohol with bubbling of N_2 gas in order to prevent the oxidation of SnCl_2 . This reagent is very unstable and should be prepared every day.

Procedure

Preparation of test solution. Add 10 ml of the 0.35 M sulfuric acid stock solution and 100 ml of isobutyl alcohol successively to 500 ml sample solutions (containing zero to 2.5 μg phosphate) in a 1000 ml separatory funnel. Shake the funnel vigorously and allow it to stand until the two phases will be completely separated (for about 30 min). After the aqueous phase is transferred to another separatory funnel, add 60 ml of the 0.012 M ammonium molybdate stock solution, shake thoroughly, and allow to stand for 15 min. Then, add 20.0 ml of isobutyl alcohol exactly, shake the funnel again vigorously for 30 to 60 s, and allow it to stand until the two phases will be sufficiently separated. Finally, add 0.2 ml of the 1 M hydrochloric acid stock solution to 5 ml of the fraction of the organic phase thus obtained.

Stopped-flow measurement. Put the prepared test solution (about 5 ml) and the color-developing reagent (about 5 ml) into each reservoir of the stopped-flow spectrophotometer. After the two solutions are rapidly mixed at a 1:1 ratio in the apparatus, record the absorbance change (ΔA) at 730 nm for about 0.5 s as a function of time. For maintaining the reducing capacity of the color-developing reagent (SnCl_2 reagent) in the reservoir, bubble N_2 gas into the reagent throughout all procedures.

Fig. 1 Typical examples of reaction curves. Phosphate concentrations: A, 2.0; B, 1.0; C, 0.5; and D, 0.0 $\mu\text{g l}^{-1}$. Other conditions were the same as those in the recommended procedures in the text.

Quantitative analysis using progress curve. The value of ΔA is determined as the difference between the absorbances at the start- and the end-points (see Fig. 1). After the value of ΔA_0 (absorbance change in the procedural blank) is determined in the same manner, $(\Delta A - \Delta A_0)$ which is proportional to phosphate concentration is calculated.

Results and Discussion

Solvent and reductant

The SFTDA technique requires a solvent which is able to dissolve not only 12MPA but also the reductant. So, isobutyl alcohol and SnCl_2 were used as the suitable solvent and reductant, respectively.^{3,13}

Stabilization of the color-developing reagent

Since SnCl_2 was much more unstable in isobutyl alcohol than in water, N_2 gas was passed through the reagent to prevent the oxidation of SnCl_2 . The reducing ability of SnCl_2 did not change over at least 5 h, even if the light was not blocked.

Periods required for formation and extraction of 12MPA

A standing-period of at least 15 min was required to form 12MPA from phosphate and molybdate under the conditions studied. And a shaking-period of more than 1 min was unnecessary in order to extract 12MPA into isobutyl alcohol. By the present extraction procedure, the efficiency of one extraction was $92 \pm 2\%$.

Stannous chloride concentration on the SFTDA method

The apparent first order rate constant (k_{app}), determined by using a Kézdy-Swinbourne plot¹⁴⁻¹⁶,

Fig. 2 Effect of SnCl_2 concentration on the absorbance change (ΔA) and the apparent first order rate constant (k_{app}). Phosphate concentration: 250 $\mu\text{g l}^{-1}$. Final (after mixing in the apparatus) HCl concentration: 0.02 M. Final SnCl_2 concentrations are given on the abscissa. ΔA (●) and k_{app} (○) were determined by using the recommended procedures in the text and the Kézdy-Swinbourne plot,¹⁴⁻¹⁶ respectively.

increases proportionally with increasing SnCl_2 concentration. The magnitude of ΔA at 730 nm apparently decreases with increasing SnCl_2 concentration, especially above 0.01 M of SnCl_2 , as shown in Fig. 2. This apparent decrease of ΔA may occur because the reactions are too fast to be followed by the stopped-flow apparatus, above 0.01 M of SnCl_2 , and the initial parts of the reaction curves are probably hidden within the dead time of the apparatus. Considering these findings, we decided to select a SnCl_2 concentration of 0.01 M (0.02 M before mixing) for sensitive and reliable measurements.

Hydrochloric acid concentration on the SFTDA method

Both the color intensity and the color-developing rate of the "Molybdenum Blue" method notably depend on the hydrogen-ion concentration (acidity).^{17,18} In the present method, HCl concentrations of about 0.02 M (0.04 M before mixing) provided the maximum value of ΔA , as shown in Fig. 3. Especially below 0.02 M HCl, ΔA decreased intensely with decreasing HCl concentration. On the other hand, k_{app} increased with decreasing HCl concentration and it showed a suitable value of about 30 s^{-1} at 0.02 M of HCl concentration. Therefore, a HCl concentration of 0.02 M was selected.

Recording period

Under the conditions studied, the recording period required for reliable measurements was only 0.5 s, which is much shorter than that required in the conventional "Molybdenum Blue" method. The reason for this difference may be that the reduction of 12MPA with stannous chloride is much faster in butanol than in water. A recording time of no longer than 0.2 s was too short to obtain suitable progress curves for reliable measurements. And that of longer than 5 s was undesirable, since the colored complex produced in the present method was very unstable and began to fade

after about 5 s from the commencement of the reaction under the conditions studied.

As shown in Fig. 1, the progress (reaction) curves obeyed well the apparent first order kinetics, and thus were different from those of the SFTDA technique combined with the "Malachite Green" method (sigmoidal progress curves).⁸ The reactions attained to the equilibrium 200 ms after the mixing.

Calibration curve

Typical examples of calibration curves for the phosphate concentrations from zero to $20 \mu\text{g l}^{-1}$ are shown in Fig. 4. Each point and vertical bars in Fig. 4(b) represent the mean $\pm$ SD (standard deviation) of four determinations. The linearity of the calibration curve was obtainable for the phosphate concentration from zero to $5 \mu\text{g l}^{-1}$.

Limit of detection

Since the SD of ΔA_0 was 0.0002 O.D., which corresponded to about $0.1 \mu\text{g l}^{-1}$ of phosphate, the detection limit was estimated to be $0.3 \mu\text{g l}^{-1}$ ($\approx 3\text{SD}$). Although the apparent molar absorptivity (about $2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$) of the present method is comparable to that (about $1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$) of our previous method⁸, this detection limit is about 1/10 that of the latter method, and is low enough for the determination of sub- $\mu\text{g l}^{-1}$ level phosphate. The reasons for this difference between detection limits are the following: Since the value of ΔA_0 is much smaller in the present method, the reaction curves can be recorded on a much more expanded scale. A lower detection limit is obtainable, if a sample volume larger than 500 μl is used.

Interference

One of the advantages of the SFTDA technique is that the slower and/or faster side reactions caused by

Fig. 3 Effect of acidity on the absorbance change (ΔA) and the apparent first order rate constant (k_{app}). Phosphate concentration: $250 \mu\text{g l}^{-1}$. Final SnCl_2 concentration: 0.01 M. Final HCl concentrations are given on the abscissa. ΔA (●) and k_{app} (○) were determined in the same manners as those in Fig. 2.

Fig. 4 Typical examples of calibration curves. ΔA was determined by the recommended procedures in the text. Each point and the vertical bars in (b) represent the mean $\pm$ SD of four determinations.

Table 1 Tolerance limit of As and Si

Ion	Added as	Tolerance limit ^a /μg l ⁻¹
As(V)	Na ₃ AsO ₄	100
As(III)	NaAsO ₂	2800
Si(IV)	Na ₄ SiO ₄	6000
Si(IV)	Na ₂ SiO ₃	5700

a. Defined as the upper limit of concentration at which an error of no more than 5% is caused, when determined at 100 μg l⁻¹ of phosphate concentration.

Table 2 Determination of phosphate in tap water and recovery test

Tap water	Added/μg l ⁻¹	Found ^a /μg l ⁻¹	Recovery, %
A	0.0	13.1±0.3	—
	20.2	33.0±0.9	98.5
B	0.0	9.2±0.2	—
	20.2	30.6±0.7	105.9
C	0.0	24.8±0.3	—
	20.2	43.7±1.2	93.6

a. Mean and standard deviation of five measurements.

contaminants can be readily excluded from the main reaction(s) to be examined.⁸ However, the interfering reactions by As(III), As(V) and Si(IV) could not be excluded, since the reaction rates of these anions and of the phosphate were very similar. The tolerance limits of these anions are shown in Table 1. As for these anions, no significant difference in their tolerance limits was found between the present method and the conventional "Molybdenum Blue" method. Therefore, for the present method to be applied to environmental water, we must discover how to remove these contaminants, especially Si(IV), by a pretreatment such as selective extraction for 12MPA, e.g., by using isobutyl acetate¹⁹ or 1-butanol:chloroform (1:4).²⁰

Application

The present method was applied to the determination of phosphate in tap waters. Table 2 shows the results of recovery tests. The relative standard deviations of five measurements on these samples are less than 3%. The values of recovery ranged between 93.6 and 105.9%.

Thus, the present method enables the precise determination of μg l⁻¹ level phosphate in water to be done with much higher (about 100 times) sensitivity and much shorter (about 1/500 times) color-developing period than conventional "Molybdenum Blue" methods.

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