2868 © 1980 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 53, 2868—2870 (1980) [Vol. 53, No. 10

Indirect Spectrophotometric Determination of Phosphate Employing the Solubilization of Methylene Blue Molybdophosphate with Zephiramine

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(Received April 4, 1980)

A highly sensitive indirect spectrophotometric method for phosphate was developed. The ion-association complex of molybdophosphate with Methylene Blue was precipitated in a nitric-acid medium. The complex was adsorbed on (1+4 v/v) cyclohexane–MIBK. The excess of Methylene Blue was removed by washing the organic phase with water. The complex was solubilized with a Zephiramine acidic aqueous solution. By measuring the absorbance of Methylene Blue in the aqueous solution at 660 nm, the phosphate was determined. The calibration curve was linear in the 0.31—3.1 μg range of phosphate, and the molar absorptivity was 2.5×10^5 l·mol^{-1}·cm^{-1}. The absorbance of the reagent blank was 0.019±0.001, and the recommended procedure was applied to the repetitive determination of 1.55 μg of phosphorus. The ten determinations produced a coefficient of variation of 1.6%. Arsenic, germanium and silicon, which form heteropolyacids like phosphorus, interfered seriously.

The studies of the solvent extraction of the ion pairs formed between complex anions and large cations are very important in analytical chemistry. Of these extractions, the one with a basic dye possessing a large molar absorptivity can be applied to the spectrophotometric determination of metals. Kirkbright *et al.*[1)] have reported a method based on the formation of a complex between Rhodamine B and molybdophosphoric acid. Golkowska and Pszonicki[2)] have studied the composition and properties of the ion-association complex of Rhodamine B with molybdosilicic acid, and have then used this method for the determination of silicon. Recently, Fogg *et al.*[3)] have reported the spectrophotometric determination of inorganic phosphate in biological systems with Crystal Violet. The absorbance characteristics of the ion-association complex formed between molybdophosphate (MoP) and Methylene Blue (MB) are similar to those of MB itself. In a previous paper,[4)] the large excess of MB co-extracted into MIBK was removed by washing it with a 2 M nitric acid solution, but the sensitivity and reproducibility were not satisfactory. This paper will report on a method for the indirect determination of phosphate by spectrophotometry. As the Methylene Blue molybdophosphate (MB·MoP) complex is only slightly soluble in the aqueous phase, it can be separated from the large excess of MB by filtration or floatation.[2)] We adopted the adsorption[5)] of the ion-association complex on the organic solvent; the MB·MoP complex precipitated as a thin layer on the phase boundary to separate from the large excess of MB. The MB^+ of the MB·MoP complex on the organic phase was replaced with the benzyldimethyltetradecylammonium ion (Zephiramine ion), and the free MB ion was transferred into the aqueous phase. By measuring the absorbance of the free MB ion in the aqueous phase, the amount of phosphate was determined.

Experimental

Apparatus and Reagents. A Hirama model 6B spectrophotometer with 10-mm glass cells was used. An Iwaki, model S-L type KM, shaker was used to shake the separatory funnel. All the chemicals were of analytical-reagent grades and were stored in polyethylene bottles. The nitric acid was distilled and diluted with water. The water was deionized and distilled.

Standard Phosphate Solution, 31 μg P ml^{-1}: Dissolve 0.1361 g of potassium dihydrogenphosphate in water and dilute to 1 l. This solution is then diluted as required to 0.31 μg P ml^{-1} with water.

Ammonium Molybdate Solution, 0.1 M: Dissolve 17.7 g of hexaammonium heptamolybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot4H_2O$ in water and dilute to 1 l.

Methylene Blue Solution, 2×10^{-3} M: Dissolve 0.747 g of Methylene Blue (Wako Pure Chemical Industries, Ltd.; 98.5% content) in water and dilute to 1 l.

Organic Solvent: A (1+4 v/v) mixture of cyclohexane and isobutyl methyl ketone(MIBK) was used.

Zephiramine Solution, 5.0×10^{-3} M: Dissolve 1.84 g of Dotite Zephiramine (Dojindo Laboratories) in water and dilute to 1 l.

Recommended Procedure. Transfer a 20-ml portion of the sample solution(previously neutralized) containing less than 3 μg P into a 100-ml separatory funnel. Add 10 ml of 4 M nitric acid, 10 ml of a 0.1 M molybdate solution, and 5 ml of a 1 M potassium nitrate solution. Allow to stand for 10 min. Then, add 1 ml of a 2×10^{-3} M Methylene Blue solution and dilute with water to 50 ml. Mix well and add about 10 ml of the organic solvent. Shake vigorously for 10 min, allow the phases to separate, and discard the aqueous phase carefully. Then, add 25 ml of water and shake for 1 min. Allow the phases to separate and discard the aqueous phase. Repeat the washing with 25 ml of water once more. Add 5 ml of a 5.0×10^{-3} M Zephiramine solution and 5 ml of 0.5 M nitric acid, and shake vigorously for 5 min. After phase separation, transfer the aqueous phase and washings into a 25-ml volumetric flask. Dilute to the mark with water. Mix well and measure the absorbance at 660 nm against a reagent blank.

Results and Discussion

Absorption Spectra. The absorption spectra of MB in the Zephiramine acidic solution obtained by the recommended procedure are shown in Fig. 1. There are shoulders at 600—620 nm and an absorption maximum at 660 nm, where the absorbance of the reagent blank is very small; that is, the absorbance is 0.019±0.001 (ten determinations).

Effects of Reaction Variables. *Concentration of Nitric Acid*: The optimum acidity of the aqueous phase for

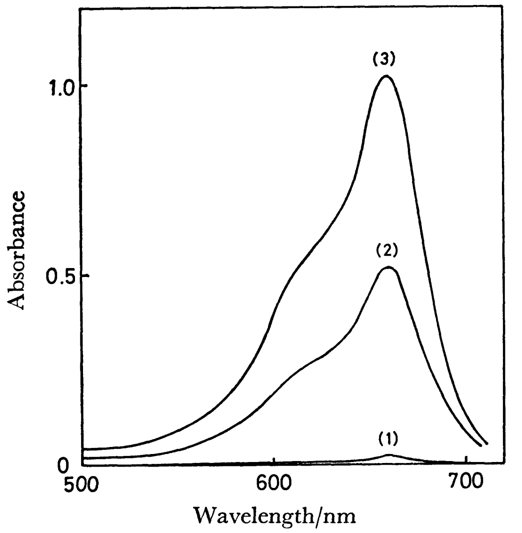

Fig. 1. Absorption spectra of Methylene Blue in Zephiramine acidic solution.
(1) Reagent blank, (2) P: 1.55 μg, (3) P: 3.10 μg. Reference: water.

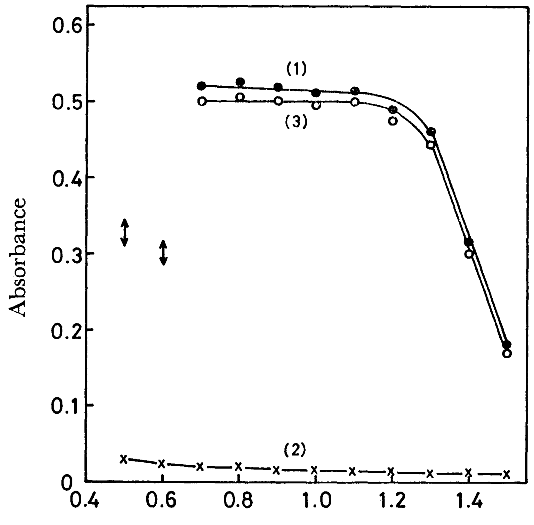

Fig. 2. Effect of nitric acid concentration on absorbance.
(1) P: 1.55 μg, (2) reagent blank, (3) net. Reference: water.

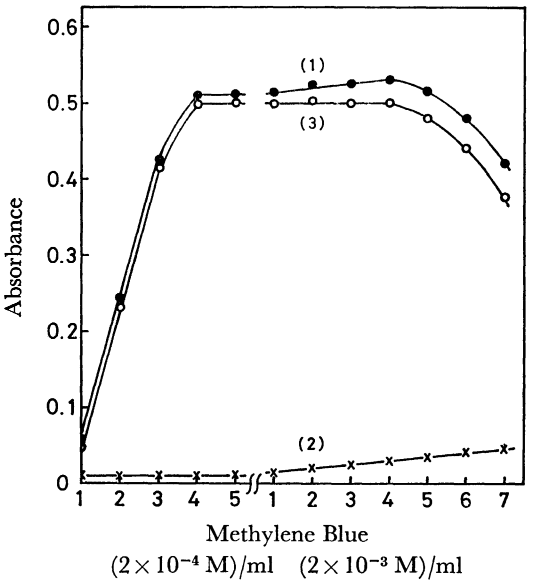

Fig. 3. Effect of the amounts of Methylene Blue solution.
(1) P: 1.55 μg, (2) reagent blank, (3) net. Reference: water.

the formation of the MB·MoP complex was found to be 0.7—1.1 M nitric acid, as is shown in Fig. 2. The absorbance decreased gradually over the 1.2—1.5 M range of nitric acid. The reproducibility was poor below 0.6 M of nitric acid, because the excess of molybdate formed a precipitate with MB. A concentration of 0.8 M nitric acid in the initial solution was, therefore, chosen as the optimum.

The Amounts of Ammonium Molybdate Solution: For 1.55 μg P the absorbance increased upon the addition of 0.1 M ammonium molybdate up to 7 ml, and then remained constant for up to 20 ml of the reagent; 10 ml of the molybdate solution was used in further work.

The Amounts of Methylene Blue Solutions: As is shown in Fig. 3, a constant and maximum absorbance was obtained when from 4 ml of a 2×10^{-4} M solution to 3 ml of a 2×10^{-3} M MB solution were added. When a MB solution below 4 ml of 2×10^{-4} M or over 3 ml of 2×10^{-3} M was added, the absorbances decreased gradually.

Concentration of Zephiramine Solution: The effect of the Zephiramine concentration in the acidic aqueous phase on the solubilization of the MB·MoP complex was also studied. The absorbance increased up to 2.5×10^{-4} M Zephiramine, this remained constant when the reagent concentration was increased further. Therefore, 2.5×10^{-3} M Zephiramine in 0.25 M nitric acid was used. Unless the acid is added in the Zephiramine solution, it is difficult to separate the phases.

Adsorption and Separation of the MB·MoP Complex.

The Choice of Organic Solvents: The considerations for the choice of the organic solvents to adsorb the MB·MoP complex are as follows: (1) the solvent should have a smaller specific gravity of the organic solvents than that of water, such a smaller specific gravity being advantageous for washing the excess of MB, (2) there should be little extractability of the MB·MoP complex, and (3) it should be possible to separate the phases rapidly after shaking. Though there are several solvents which satisfy these conditions, the mixture of MIBK and cyclohexane was most recommended.

Effect of the Addition of Cyclohexane to MIBK: As is shown in Fig. 4, the optimum range was 20—30% (v/v) cyclohexane in MIBK, at which the absorbance of the reagent blank was very small. The emulsion forms in a yield of more than 40% upon shaking with the Zephiramine solution. Therefore, the shaking was carried out at 20%; that is, a (1+4) cyclohexane–MIBK mixture was used in further work.

Volume of (1+4)Cyclohexane-MIBK: The effect of (1+4) cyclohexane-MIBK was examined by varying the volume from 5 to 20 ml according to the recom-

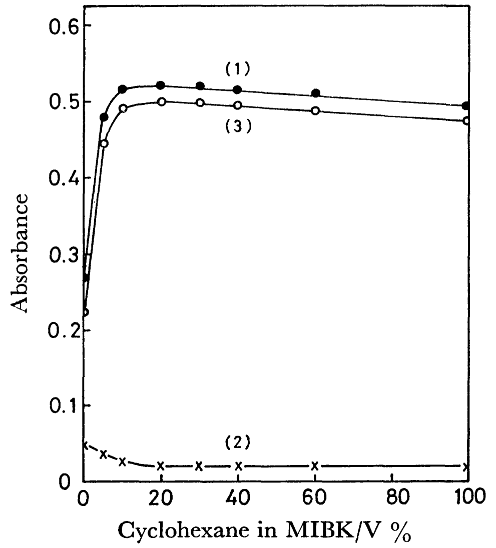

Fig. 4. Effect of the addition of cyclohexane.
(1) P: 1.55 μg, (2) reagent blank, (3) net. Reference: water.

mended procedure. However, no effect of the volume was observed. Therefore, about 10 ml of the solvent was used in further work.

Washing the Organic Phase: To remove the large excess of MB after the adsorption of the MB·MoP complex, we washed the organic phase with water. Washing twice with 25-ml portions of water was sufficient to remove the excess reagent. The shaking time for washing was 1 min.

Calibration Curve, Sensitivity, and Precision. The calibration curve obtained by the recommended procedure was linear in the range from 0.31 to 3.1 μg of phosphorus, and the molar absorptivity, as calculated from the slope of the curve, was 2.5×10^5 l·mol^{-1}·cm^{-1}. The recommended procedure was applied to the repetitive determination of 1.55 μg of phosphorus. The ten determinations produced a coefficient of variation of 1.6%.

Composition of the MB·MoP Complex. When the MB·MoP complex was dissolved with the Zephiramine solution, it was found that MB was transferred into the aqueous phase and that MoP was extracted into the organic phase. The absorbance of free MB in the aqueous phase was measured, and the concentration of MB was determined using the calibration curve of free MB. The P: MB ratio was 1: 3. After removing the organic solvent by volatilization, the analysis of MoP for molybdenum by atomic-absorption spectrophotometry, and that for phosphorus by the molyddenum blue method,[6] gave these results, P : Mo = 1 : 12. The composition of the MB·MoP complex was estimated as $(MB)_3PMo_{12}O_{40}$.

Table 1. Effect of diverse ions on the recovery of phosphorus

Ion	Added as	Amount (μg)	Recovery (%)
As(V)	$Na_2HAsO_4 \cdot 7H_2O$	0.15	103
		1.0	119
As(III)	As_2O_3[a]	5.0	103
		20	112
Ge(IV)	GeO_2[a]	0.50	102
		5.0	122
Si(IV)	SiO_2[a]	20	102
		200	122
V(V)	NH_4VO_3	2.0	104
		40	110
Cr(VI)	$K_2Cr_2O_7$	15	98
		200	87

a) Treated with NaOH. P: 1.55 μg.

Effect of Diverse Ions. The effect of diverse ions on the recovery produced from 1.55 μg of phosphorus by the recommended procedure was investigated. An ion was considered not to interfere when the error in the recovery was less than ±4% for the test solutions compared with the standards. As is shown in Table 1, arsenic, germanium and silicon, which form heteropolyacids like phosphorus, interfered seriously. These results are in agreement with those of a previous paper.[4]

Application. The method described may be used for the microanalysis of phosphate, for example, sea water and river water. Because the MB·MoP complex dissolves in alcohols (methanol, ethanol, *etc.*) or amides (*N*,*N*-dimethylformamide, *etc.*), phosphorus can be determined directly by measuring the absorbance of the complex in an organic solvent. Therefore, this makes possible a considerable reduction in time.

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