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Spectrophotometric Determination of Phosphate Ion with Ferric Salt of Tetraphenylporphine-trisulfonic Acid[1]

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A new indirect spectrophotometric method for the determination of phosphate ion by the use of solid ferric salt of tetraphenylporphine-trisulfonic acid (TPPSFe) was developed. The method is based on the measurement of the absorbance of TPPS liberated from TPPSFe at its Q band (procedure A) or its Soret band (procedure B). Phosphate ion ranging from 10 to 50 μg and from 4 to 20 μg could be determined based on the absorption coefficients of 8.2×10^3 at 512 nm (procedure A) and 2.2×10^5 at 434 nm (procedure B). The advantages of this method are its simple procedure and mild conditions. Procedure A was successfully applied to the determination of phosphate ion liberated from glucose-1-phosphate by acid phosphatase.

Keywords——indirect spectrophotometry; determination of phosphate ion; tetraphenylporphine-trisulfonic acid; ferric salt; glucose-1-phosphate; acid phosphatase

The widely used phosphomolybdate method[2] and an improved method[3] for the determination of phosphate ion both require a reducing agent and strong acid, and are not always satisfactory. Some water-soluble porphines have been widely applied to the determination of trace amounts of metal ions based on their extremely large molar absorptivity.[4] However, their application to the determination of anions has not been attempted. We have developed a method for the determination of phosphate ion by the use of the easily obtainable ferric salt of tetraphenylporphine-trisulfonic acid (TPPSFe), which may be present as a polymer and does not contain ferric ion in its porphine ring. TPPSFe is slightly water-soluble and liberates tetraphenylporphine-trisulfonic acid (TPPS) on reaction with phosphate ion; thus, phosphate ion can be determined spectrophotometrically based on the amount of TPPS released. The reaction may be expressed as follows.

$$\mathrm{TPPSFe(III)} + \mathrm{PO_4^{3-}} \longrightarrow \mathrm{TPPS^{3-}} + \mathrm{FePO_4} \qquad (1)$$

Experimental

Materials——TPPS was prepared by the sulfonation of tetraphenylporphine according to the method of Alder *et al.*,[5] and purified by reprecipitation from concentrated hydrochloric acid. An aqueous solution of $\mathrm{FeCl_3 \cdot 6H_2O}$ (0.5 g) was added to TPPS (0.5 g) aqueous solution with stirring, and the precipitate was centrifugated, filtered off, washed with water and dried over P_2O_5 *in vacuo*. TPPSFe reagent was prepared by the suitable dilution with lactose. Dioxane was distilled and dried with molecular sieves 4A (Wako Junyaku Ltd.). Other reagents were of analytical or reagent grade. A clinical kit based on the formation of phosphomolybdate complex (Phosphor B-test *Wako*, Wako Junyaku Ltd.) was used to obtain the control value of phosphate ion.

Apparatus——A Shimadzu UV-180 double beam spectrophotometer with 10 mm quartz cells was used for the measurement of absorbance. A Hitachi-Horiba M-5 pH meter was used for pH measurements.

Recommended Procedures——Procedure A: Acetate buffer (0.1 M, pH 4.0, 0.02 ml), dioxane (5 ml) and 10% TPPSFe reagent (5 mg) were added to the sample solution (0.5 ml, 10—50 μg of $\mathrm{PO_4^{3-}}$), and the mixture was shaken for 30 min. Remaining TPPSFe was removed by centrifugation for 20 min. The absorbance of the supernatant was measured at 512 nm against the reagent blank.

Procedure B: A mixture of sample solution (0.2 ml, 4—20 μg of PO_4^{3-}), buffer solution (0.01 ml), dioxane (2.0 ml) and 2% TPPSFe reagent (5 mg) was treated in the same manner as in procedure A. The resulting solution was centrifuged twice and 0.2 ml of the supernatant was taken and added to 0.1 M acetate buffer (pH 3.5, 4.0 ml). The absorbance of the diluted solution was measured at 434 nm against the reagent blank.

Results and Discussion

Absorption Spectra

In Fig. 1, the absorption spectra of the solutions obtained by procedures A and B are presented. These spectra coincided with its spectrum of TPPS solution, and the liberation of TPPS based on the reaction (1) was confirmed. TPPS showed Q bands at 512, 547, 588 and 645 nm in a mixture of water and dioxane. In the present study, we selected the Q band at 512 nm and the Soret band at 434 nm for procedures A and B, respectively.

Selection of Reaction Conditions

The conditions for procedures A and B were examined for 40 μg of phosphate ion, and 16 μg of phosphate ion, respectively.

Organic Solvent——The reagent blank was too large to permit determination in an aqueous system, in spite of the low solubility of TPPS. In order to decrease the reagent blank value, the addition of dioxane, butanol, dimethylsulfoxide, methanol, ethanol or isopropanol was examined. Among these solvents, only dioxane gave a satisfactory result. The absorbances of the reagent blank and the colored solution decreased with increasing ratio of water to dioxane. The addition of dioxane to give a ratio of the sample solution to dioxane of 1 : 10 was found to be adequate to obtain good reproducibility and linearity of the calibration curve.

Effect of pH——As can be seen in Fig. 2, although the absorbance of the reagent blank and the colored solution increased with the increase of the pH, the absorbance difference was maximum at around pH 4.0.

Effect of Buffer Concentration——The addition of 0.1 M acetate buffer was found to be adequate, because the maximum absorbance difference was obtained when 0.1—0.15 M acetate buffer was used. When more than 0.2 M acetate buffer was added, the absorbances increased remarkably, whereas the absorbance difference decreased.

Effects of Amount and Dilution of TPPSFe——The absorbance of the colored solution

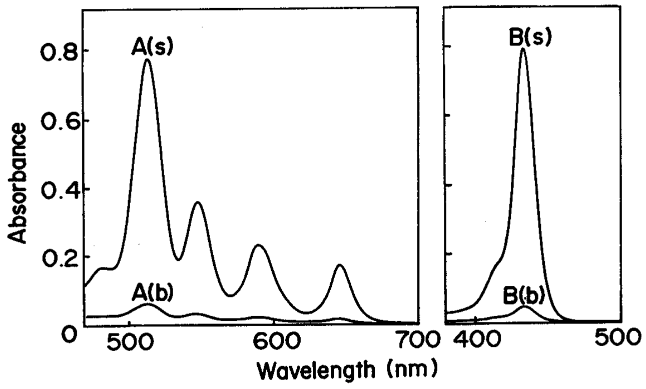

Fig. 1. Absorption Spectra

A(b), reagent blank of procedure A; A(s), colored solution of procedure A (7.25 μg/ml PO_4^{3-}); B(b), reagent blank of procedure B; B(s), colored solution of procedure B (0.35 μg/ml PO_4^{3-}).

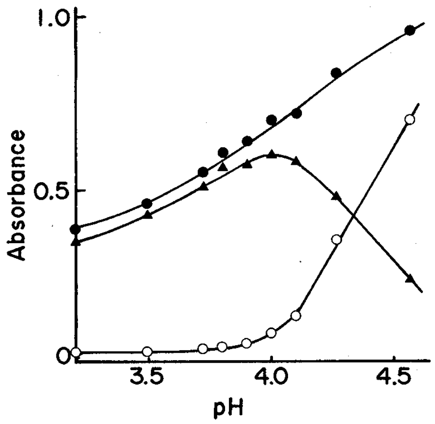

Fig. 2. Effect of pH

—○—, reagent blank; —●—, colored solution; —▲—, absorbance difference.

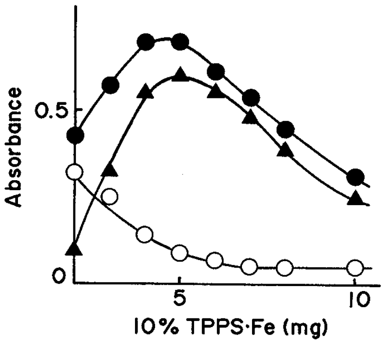

Fig. 3. Effect of Amount of 10% TPPSFe

—○—, reagent blank; —●—, colored solution; —▲—, absorbance difference.

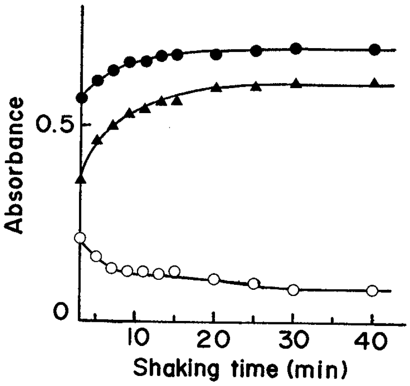

Fig. 4. Effect of Shaking Time

—○—, reagent blank; —●—, colored solution; —▲—, absorbance difference.

and the difference from that of the blank were maximum with 5 mg of TPPSFe, as shown in Fig. 3. An increase of the amount of TPPSFe caused a decrease of the absorbance, probably because remaining TPPSFe adsorbed liberated TPPS.

The degree of the dilution of TPPSFe with lactose did not significantly affect the absorbances in the range between 5 to 15%. As the diluent, lactose was found to be the best among various substances tested, such as $FePO_4$, starch and talc.

Effect of Shaking and Centrifugation Time——The absorbance difference was found to be constant when the shaking time was longer than 20 min, as shown in Fig. 4.

Centrifugation for 20 min was found to be enough to separate remaining TPPSFe. When filtration with filter paper was applied instead of centrifugation, TPPS liberated was adsorbed completely on the paper.

Dilution of the Colored Solution for Procedure B——Under conditions similar to those of procedure A, the absorbance of the Soret band of the reagent blank was too large. A change of pH, dilution of the colored solution by the solvent and the addition of excess dioxane may decrease the reagent blank value. We examined the effect of dilution with dioxane, water and pH 3.5 and 5.0 buffer solution. The addition of pH 3.5 buffer solution gave a good result. For procedure B, two centrifugations were necessary, and the use of 2% TPPSFe in lactose gave good reproducibility.

Calibration Curves, Sensitivity and Reproducibility

The calibration curves obtained by procedures A and B showed good linearity. The molar absorption coefficients were 8.2×10^3 and 2.2×10^5 for procedures A and B, respectively. These values correspond to approximately half of the molar absorption coefficient of TPPS. It appears that the reaction between PO_4^{3-} and TPPSFe proceeds consistently under the reaction conditions used.

The sensitivity of procedure A is comparable with that of the conventional molybdate blue method, namely the clinical kit method (Phosphor B-test *Wako*) or Goldenberg's method.[6] The sensitivity of procedure B is more than ten times that of the control molybdate blue method, and is comparable to that obtained with the extraction method reported by Motomizu *et al.*[7]

Coefficients of variation of procedures A and B (2.3 and 3.4%, respectively) were satisfactory, even though the method is based on a solid-solution reaction.

Interference

The interference by anions such as Cl^-, Br^-, NO_3^-, CO_3^{2-} and SO_4^{2-} was tested. Only CO_3^{2-} ion showed significant interference. The metal ions which form a water-insoluble salt with PO_4^{3-} caused a large decrease in absorbance. This interference could be removed by treatment with cation-exchange resin.

Application

Procedure A gave a good result in the determination of phosphate ion liberated from glucose-1-phosphate by acid phosphatase. A mixture of glucose-1-phosphate (0.2 ml, 15—45 μg PO_4^{3-}), acetate buffer (0.05 M, pH 4.0, 0.1 ml) and acid phosphatase solution (0.4 U, 0.2 ml) was incubated for 10 min at 37 °C, and the incubated mixture was used as a sample solution.

Conclusion

The method described above may be conveniently applied to the determination of phosphate ion liberated enzymatically from organic phosphates. The advantages of this method are that the reaction conditions are mild and no extraction procedure is necessary. The results presented here indicated the extremely high molar absorptivity of porphines may be applicable to the determination of some anions, as well as of metal ions.

References and Notes

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