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Application of *o*-Hydroxyhydroquinonephthalein–Iron(III) Complex to Determination of Organic Compounds Containing Phosphorus[1)]

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An application of *o*-hydroxyhydroquinonephthalein–iron(III) complex to the determination of organic compounds containing phosphorus, such as adenosine 5′-triphosphate (ATP), was developed. In the determination of ATP, Beer's law held up to 4 μg of ATP in the final volume of 10 ml, the apparent molar absorptivity being 1.1×10^6 dm^3 mol^{-1} cm^{-1}. Other compounds containing phosphorus such as glucose-6-phosphate, phytic acid and L-α-lecithin could also be determined under the standard conditions. The proposed method, in which the use of buffer and surfactant solutions was modified, is relatively simple, does not require preliminary treatment and is about 5 times more sensitive than previous method.

Keywords——organophosphorus compound; spectrophotometry; *o*-hydroxyhydroquinonephthalein; iron(III); adenosine 5′-triphosphate; surfactant; cetylpyridinium chloride; polyvinyl pyrrolidone

In general, the determination of organic compounds containing phosphorus, such as phospholipids and nucleotides, is done by measuring free phosphate ion after destroying the organic compounds or phosphorus derivatives through preliminary treatment.[2–5)]

We have already reported[6)] the sensitive spectrophotometric determination of phosphorus (as orthophosphate ion) by using *o*-hydroxyhydroquinonephthalein (Qnph) and iron(III), and suggested that it might be possible to determine directly organic compounds containing phosphorus, such as adenosine 5′-triphosphate (ATP) and flavin adenine dinucleotide (FAD).

In this work, we describe a direct method, which is greatly improved with respect to sensitivity, for the determination of organic compounds containing phosphorus by using Qnph–iron(III) complex.

Experimental

Reagents, Materials and Apparatus——ATP (assay, 99%), adenosine 5′-diphosphate (ADP, assay, 99%), adenosine 5′-monophosphate (AMP, assay, 98%), β-nicotinamide adenine dinucleotide (β-NAD, assay, 98%), FAD (assay, 90%), D-glucose-6-phosphate (assay, 98%), creatine phosphate (assay, 99%), phytic acid and L-α-phosphatidylcholine (L-α-lecithin, obtained from soybean) were purchased as the free acids or sodium salts from Oriental Yeast Co., Ltd., Kojin Co., Ltd., Sigma Chem. Co., Nakarai Yakuhin Co., and Tokyo Kasei Kogyo Co., Ltd. The methods of preparation of Qnph and iron(III) solutions and the apparatus used were as described in the previous report.[6)] All other reagents and materials were of reagent grade, and deionized water was used.

Recommended Procedure——For the determination of ATP: A solution containing up to 4 μg of ATP was added to a 10-ml (ml = cm^3) volumetric flask. To this solution were added 0.4 ml of a 5.0×10^{-4} M (M = mol cm^{-3}) iron(III) solution, 0.25 ml of a 1.0×10^{-2} M cetylpyridinium chloride (CPC) solution, 0.75 ml of a 2.0% polyvinyl pyrrolidone (PVP) solution, 2.5 ml of a 0.1 M ammonia–0.1 M ammonium chloride solution (pH 9.4) and 0.5 ml of a 1.0×10^{-3}M Qnph solution. The mixture was diluted to 10 ml with water, kept at 60 °C for 45 min, and cooled to room

temperature. The absorbance of the Qnph–iron(III)–ATP solution (solution A) was measured at 630 nm against the Qnph–iron(III) solution (solution B).

Results and Discussion

ATP, which is representative of organic compounds containing phosphorus, was chosen for the development of appropriate conditions for the determination of organic compounds containing phosphorus.

Figure 1 shows the absorption spectra of solutions A, B and Qnph solution (solution C) under the standard conditions. The absorption maximum of solution A against solution B was at about 630 nm. Constant absorbance was obtained between pH 9.0 and 9.8. The 0.1 M ammonia–0.1 M ammonium chloride buffer solution was found to be satisfactory for pH adjustments. On the other hand, the use[6] of borax buffer solution had some disadvantages: Qnph tended to decompose in light, solutions A and B were fairly unstable, and the absorbance of solution B was extremely high.

Among various surfactants examined, CPC, a cationic surfactant, was most effective with respect to sensitivity. However, the use of CPC alone required more than 90 min at 60 °C for complete color development, and the reproducibility of absorbance of solutions A and B was poor. This disadvantage could be to some extent eliminated by using PVP (a nonionic

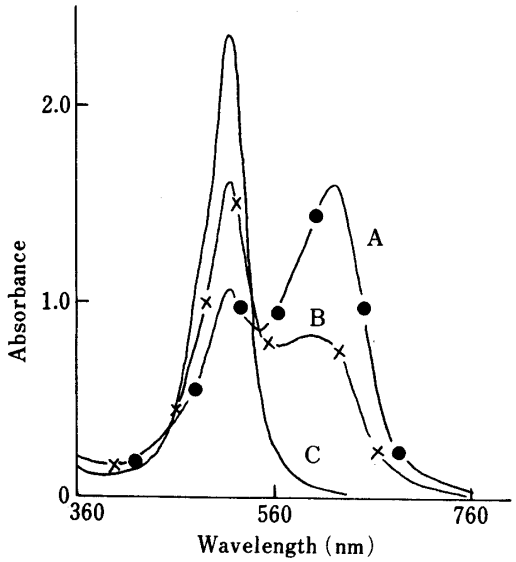

Fig. 1. Absorption Spectra of Solutions A, B and C in the Recommended Procedure

Curve A, solution A [Qnph–iron(III)–ATP]; curve B, solution B [Qnph–iron(III)]; curve C, solution C [Qnph]; ATP, 3.5 μg/10 ml; reference, water.

Table I. Apparent Molar Absorptivities for Various Organic Compounds Containing Phosphorus

Compounds	ε[a] ($dm^3\ mol^{-1}\ cm^{-1}$)
ATP	1.1×10^6
ADP	8.6×10^5
AMP	7.1×10^5
β-NAD	4.1×10^5
FAD	1.8×10^5
Cocarboxylase	1.8×10^5
Glucose-6-phosphate	2.0×10^5
Creatine phosphate	1.7×10^5
Phytic acid	2.4×10^6
L-α-Lecithin	(*ca.* 50 μg)[b]

a) Apparent molar absorptivity (uncorrected). *b*) As molecular weight and concentration were uncertain, the approximate range of application is shown.

Table II. Analytical Results for ATP in Commercial Tablets

Samples	ATP·2Na, amount		Recovery[b] (%)	C.V.[c] (%)
	Manifested (mg)	Found[a] (mg)		
A	20	20.3	103.2	2.5
B	20	20.0	101.7	2.5

a) Average of 3 determinations. *b*) ATP taken, 1.5 μg/10 ml. *c*) Coefficient of variation.

surfactant) solution together with CPC. The maximum absorbance was obtained by heating these solutions at 60 °C for 45 min then cooling to room temperature. The absorbance was constant for at least 2 h.

In the determination of ATP, Beer's law held in the range up to 4 μg of ATP in the final volume of 10 ml. The apparent molar absorptivity and the coefficient of variation were $1.1 \times 10^6 dm^3 mol^{-1} cm^{-1}$ and 2.3% ($n=5$) for 2.5 μg of ATP. Table I shows the approximate apparent molar absorptivities of various organic compounds containing phosphorus. It seems likely that the apparent molar absorptivities are correlated with the number of phosphorus atoms in the molecule.

The iron(III)-to-Qnph ratio and the ATP-to-iron(III) ratio as determined by the molar ratio and continuous variation methods were 1 : 2 and 1 : 3, respectively. Thus, the colored species formed among Qnph, iron(III) and ATP may be expressed as [ATP 1 : iron(III) 3 : Qnph 6]. The hydrolysis of ATP is slow under the conditions described above, and the reaction among phosphate ion, iron(III) and Qnph shows the same color, so the chromogen in this color reaction system may be phosphate ion and/or phosphate ester. Further investigation of the composition and species of the chromogen is necessary.

Under the standard conditions, the interference by various ions and substances with the determination of 2.5 μg of ATP was studied. Most metal ions interfered very little when present in 2-fold molar excess over ATP. Among the anions tested, phosphate ion and hydroxypolycarboxylate ions such as citrate and tartrate gave positive errors[6,7] even when present at one-half molar concentration with respect to ATP. The effects of these interfering ions could be minimized by a standard addition method. Other anions, D-glucose, amino acids, adenine, adenosine and some drugs such as caffeine, cephalexin, thiamine, *etc.* did not interfere when present at 20- to 200-fold molar excess over ATP. The coexistence of up to 20 μg of albumin(human) had no effect.

The proposed method was applied to the assay of ATP in commercial tablets. The recoveries of ATP from the sample solutions were about 101—103%, as shown in Table II.

Though further development is necessary, this method, in which the use of buffer and surfactant solutions was modified, is about 5 times more sensitive than that described in the previous report,[6] is relatively simple, and requires no preliminary treatment. The proposed method may be useful for the determination and detection of various organic compounds containing phosphorus, such as phospholipids and nucleotides.

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