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The Spectrophotometric Determination of Phosphorus with *o*-Hydroxyhydroquinonephthalein and Iron(III)†

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On this basis of the coloration formed with *o*-hydroxyhydroquinonephthalein, an anionic dye, and iron (III) in aqueous media, trace amounts of phosphorus(as orthophosphate ion) was determined. A sample solution containing posphorus and the reagent blank in an amber-colored test tube with a stopper were heated at 40 °C for 20 min in the presence of poly(oxyethylene)sorbitan monolaurate, a nonionic surfactant, as a dispersion agent. Beer's law was obeyed over the range of ≈1 μg/10 cm^3 of phosphorus, and the apparent molar absorptivity of the complex was 2.5×10^5 dm^3 mol^{-1} cm^{-1} at 610 nm. The present method was applied to the determination of total phosphorus in water — a sample solution containing small amounts of metal ions —, and the recovery percentage was satisfactory, about 95%. Other compounds containing phosphorus such as 5′-adenosine triphosphate could also be determined without a preliminary treatment.

Recently, various sensitive spectrophotometric methods for the determination of phosphorus have been reported.[1-19] Most of these methods are based upon the formation of heteropoly acids such as molybdophosphate ion, or the ionic-association complex formation with cationic dyes such as Crystal Violet,[4-7] Ethyl Violet(EV),[8,9] Malachite Green(MG),[10-14] Methylene Blue,[15] Safranin T[16,17] and Rhodamine B.[18,19] However, little work has been done on the determination of phosphorus by using an anionic dye, though an indirect method[20,21] has been studied by utilizing the coloration between molybdenum(VI) freed from heteropolyacid and Pyrocatechol Violet (PV).

Meanwhile, we noticed that the addition of micro amounts of phosphorus (as orthophosphate ion) to the reaction mixture between iron(III) and *o*-hydroxyhydroquinonephthalein(Qnph), an anionic dye, produced a different spectrum than that of the Qnph-iron(III) complex solution in a weakly basic medium.

In this paper, optimum conditions for a new spectrophotometric determination of micro amounts of phosphorus using Qnph and iron(III) were studied, and phosphorus as well as compounds containing phosphorus in water was determined.

Experimental

Reagents and Materials. A standard solution (1.0×10^{-2} mol dm^{-3}) of phosphorus was prepared by dissolving a proper quantity of potassium dihydrogenphosphate (Merck) in water, which was dried at 110 °C to a constant weight. The working solution (5.0×10^{-5} mol dm^{-3}) was made by diluting this stock solution as required. A Qnph solution (1.0×10^{-3} mol dm^{-3}) in methanol was prepared by dissolving Qnph synthesized by the published procedure.[22] An iron(III) solution (5.0×10^{-4} mol dm^{-3}) was prepared by dissolving a proper quantity of iron(III) ammonium sulfate in 5 cm^3 of 1.0% sulfuric acid and diluting the mixture to 100 cm^3 with water. A 5.0% poly(oxyethylene)sorbitan monolaurate (Nihon Yushi Co. Ltd., LT-221) solution was prepared by dissolving it with water. A solution (1.0×10^{-1} mol dm^{-3}) of sodium tetraborate(borax) was prepared as a buffer solution. All the other reagents and materials were of an analytical grade and were used without further purification. Doubly distilled water was used.

Apparatus. A Shimadzu Model UV-240 recording spectrophotometer with 1.0-cm quartz cells was used for the absorption spectrum and absorbance measurements. A Hitachi-Horiba Model F-7 AD glass electrode pH meter was used for the pH measurements.

Standard Procedure for the Determination of Phosphorus. To a solution containing up to 1 μg phosphorus in a 10 cm^3 volumetric flask were added 1.0 cm^3 of a 5.0% LT-221 solution, 0.5 cm^3 of a 5.0×10^{-4} mol dm^{-3} iron(III) solution, 4.0 cm^3 of a 1.0×10^{-1} mol dm^{-3} borax solution, and 0.5 cm^3 of a 1.0×10^{-3} mol dm^{-3} Qnph solution. The mixture was diluted to 10 cm^3 with water, and the solution was transferred to an amber-colored test tube with a stopper, kept at 40 °C for 20 min and then cooled to room temperature. The absorbance of the Qnph-iron(III)-phosphorus solution (Solution A) was measured at 610 nm against the Qnph-iron(III) solution(Solution B).

Results and Discussion

Color Reaction and Absorption Spectra. The addition of the phosphorus solution to Solution B exhibited an extreme increase in absorbance, and its absorbance at around 610 nm was proportional to the concentration of phosphorus. The reproducibility of color development of Solutions A and B without a surfactant was unfavorable. The use of a nonionic surfactant such as LT-221 was effective as a dispersion agent.

The effect of dyes in this reaction system was examined by measuring the difference of absorbance between a dye-iron(III)-phosphorus solution and a dye-iron(III) solution. The use of xanthene dyes such

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as Qnph, phenylfluorone(Phfl), Pyrogallol Red(PR) and gallein(Gall) enhanced the absorbance of the dye-iron(III) solution. The reaction sensitivity among Qnph, Phfl, PR, and Gall decreased in the sequence: Qnph≧Phfl ≫ PR and Gall. As for other dyes such as Xylenol Orange, Chromazurol S, PV, and 1-(2-pyridylazo)-2-naphthol, no difference of absorption spectra between the dye-iron(III)-phosphorus solution and the dye-iron(III) solution was observed under the standard conditions.

The effect of metal ions was also examined. Iron(III) or iron(II) ion was effective among the various metal ions tested: iron(III), iron(II), copper(II), aluminum (III), cerium(III), manganese(II), thorium(IV), molybdenum(VI), vanadium(V), cobalt(II), *etc.* Iron(III) was fairly superior to iron(II) in terms of sensitivity.

On the basis of these results, Qnph and iron(III) were chosen for the purpose of fundamental investigations on the determination of phosphorus.

The absorption spectra of Solution A, Solution B and Qnph solution under the standard conditions are shown in Fig. 1.

Effect of pH. The effect of pH on the color development was investigated with a solution containing 0.62 μg/10 cm^3 phosphorus. The maximum and nearly constant absorbance was observed at pH 9.0—9.6. A borax buffer was most effective in terms of sensitivity among the various buffer solutions, such as borax, ammonia-ammonium chloride and carbonate-hydrogencarbonate solutions. In addition, the color reaction between Qnph and various metal ions in borax solution was limited than that in the other buffer solutions, and the influence of other metal ions was relatively small. The constant absorbance was observed upon the addition of more than 3.0 cm^3 of 1.0×10^{-1} mol dm^{-3} borax in the final volume of 10 cm^3. Accordingly, 4.0 cm^3 of 1.0×10^{-1} mol dm^{-3} borax solution (pH 9.3) was used as the buffer solution.

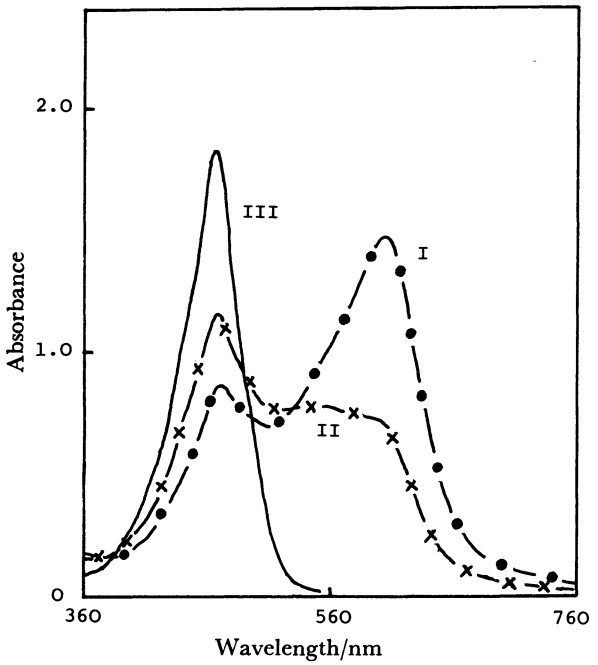

Fig. 1. Absorption spectra of Qnph–iron(III)-phosphorus, Qnph-iron(III) and Qnph solutions.
Phosphorus: 3.0×10^{-6} mol dm^{-3}; iron(III): 2.5×10^{-5} mol dm^{-3}; Qnph: 5.0×10^{-5} mol dm^{-3}; LT-221: 1.0 cm^3 of 5.0% LT-221 solution/10ml; borax: 4.0×10^{-2} mol dm^{-3}; Reference: water; curve I: Qnph–iron (III)-phosphorus solution; curve II: Qnph–iron(III) solution; curve III : Qnph solution.

Effect of Surfactants. Among various surfactants tested, LT-221, a nonionic surfactant, was best as a dispersion agent; the maximum and constant absorbance of Solution A against Solution B was observed upon the addition of more than 0.5 cm^3 of a 5.0% LT-221 solution to the final volume of 10 cm^3. The results are given in Table 1.

Effects of Qnph and Iron(III) Concentrations. The effects of the amounts of Qnph and iron(III) were examined by varying the molar ratio of iron(III) to Qnph, the amount of phosphorus being kept constant. As is shown in Fig, 2, the maximum and constant absorbance at 610 nm was observed upon the addition of a 0.4—0.6 cm^3 of 5.0×10^{-4} mol dm^{-3} iron(III) solution in the final volume of 10 cm^3. By the way, the molar ratio of iron(III) to Qnph in the reaction system was found to be 1:2 by the molar-ratio method in the presence of phosphorus. Accordingly, all further work was carried out with 2.5×10^{-5} mol dm^{-3} iron (III) and 5.0×10^{-5} mol dm^{-3} Qnph in the final volume.

Temperature and Stability. The absorbance of Solution A at 610 nm against Solution B was

Table 1. Effect of surfactants

Surfactants	Absorbance at	λ_{max}
None	0.250	610
LT-221	0.495	610
Poly(oxyethylene)sorbitan monooleate	0.475	610
Polyethylene glycol mono[*p*-(6-methylheptyl)phenyl]ether	0.490	610
Poly(vinyl alcohol)(*n*: 500)	0.465	610
Poly(*N*-vinylpyrrolidone) (K-30)	0.235	615
Gum arabic	0.028	600
Sodium dodecylsulfate	0.375	595
Sodium dodecylbenzenesulfonate	0.028	590
Hexadecyltrimethylammonium chloride	0.225	620
Benzyldimethyltetradecylammonium chloride	0.180	620

P taken: 0.62 μg/10 cm^3; Fe(III): 2.5×10^{-5} mol dm^{-3}; Qnph: 5.0×10^{-5} mol dm^{-3}; surfactant: 2.0 cm^3 of 2.0% surfactant solution/10 cm^3; borax: 4.0×10^{-2} mol dm^{-3}; Reference: Solution B.

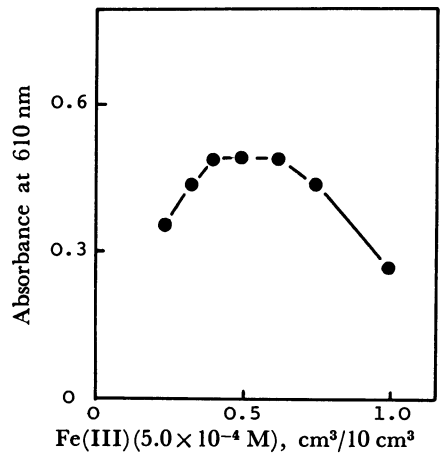

Fig. 2. Effect of iron(III) concentration.
Phosphorus: 2.0×10^{-6} mol dm^{-3}(0.62 μg/10 cm^3); Qnph: 5.0×10^{-5} mol dm^{-3}; LT-221: 1.0 cm^3 of 5.0% LT-221 solution/10 cm^3; borax: 4.0×10^{-2} mol dm^{-3}; Reference: Solution B.

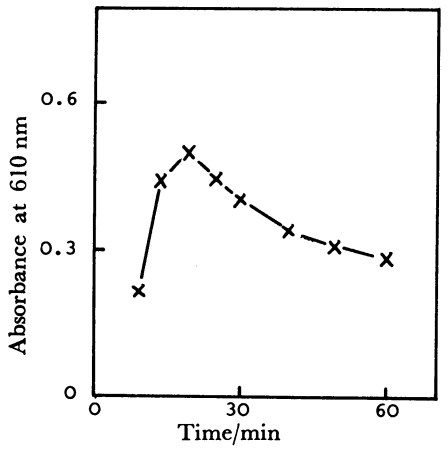

Fig. 3. Relationship between absorbance and heating time at 40 °C.
Phosphorus: 2.0×10^{-6} mol dm^{-3}; iron(III): 2.5×10^{-5} mol dm^{-3}; Qnph: 5.0×10^{-5} mol dm^{-3}; LT-221: 1.0 cm^3 of 5.0% LT-221 solution/10cm^3; borax: 4.0×10^{-2} mol dm^{-3}; Reference: Solution B.

unconstant instantaneously at room temperature (15—25 °C), and more than 6 h was required to obtain a nearly constant absorbance. The effect of the temperature was examined by heating the mixed solution for 10—60 min at various temperatures (35 °C, 40 °C, 45 °C, or 50 °C); the favorable result was obtained by heating the solution at 40 °C for 20 min and then cooling it to room temperature. The relationship between absorbance and heating time at 40 °C is shown in Fig. 3.

On the other hand, Solutions A and B were found to be fairly unstable and lacked reproducibility in diffused light. The reason was probably that Qnph in borax solution easily decomposed by light. The disadvantage is conveniently improved by the use of an amber-colored test tube.

From the results described above, the absorbance of Solution A at 610 nm against Solution B was almost constant for at least 3 h at room temperature under the standard conditions.

Calibration Curve and Repeatability. The calibration curve for phosphorus was constructed according to the standard procedure. Beer's law held in the concentration range of ≈1 μg of phosphorus in the final volume of 10 cm^3. The apparent molar absorptivity at 610 nm was estimated to be 2.5×10^5 dm^3 mol^{-1} cm^{-1}, and the Sandell sensitivity was calculated to be 0.00013 μg cm^{-2} for phosphorus. The coefficient of variation (n=8) was 2.5% for 0.62 μg of phosphorus.

Effects of Foreign Ions. The effects of foreign ions on the determination of phosphorus were examined. Among metal ions tested, small amounts of vanadium(V), zirconium(IV) and copper(II) interfered, but iron(III), aluminum(III) and arsenic(V) did not interfere in equimolar amounts with respect to phosphorus. Divalent heavy metal ions such as manganese(II), cobalt(II), zinc(II) and lead(II) did not interfere in the 5- to 10-fold excess over phosphorus, and other metal ions such as magnesium(II) and calcium(II) did not interfere in the 100-fold excess. Among various anions tested, the presence of small amounts of organic acids such as tartrate and citrate ions, and large amounts of oxalate, thiocyanate, thiosulfate and cyanide ions interfered. Other anions such as nitrate, chloride, and sulfate ions scarcely affected the determination of phosphorus. However, when strong electrolytes were present more than 5000-fold molar excess of phosphorus, it was necessary to measure the difference of absorbance between Solutions A and B containing the same ingredients. The results are summarized in Table 2.

The Composition of the Complex. The molar ratio of iron(III) to Qnph in the presence of phosphorus was estimated by the molar-ratio and continuous-variation methods. The results indicated that the molar ratio of iron(III) to Qnph was 1:2. The result obtained by the continuous-variation method is shown in Fig. 4. The phosphorus-to-iron(III) ratio obtained by the molar-ratio method was 1:2, as is shown in Fig. 5. From the results, the ternary complex formed in this reaction system is expressed as [phosphorus 1: iron(III) 2: Qnph 4].

Application for the Determination of Phosphorus in Water. The present method was applied to the determination of phosphorus in water (effluent of treated waste water and rain water). On the other hand, orthophosphate ion and total phosphorus in the same samples were determined according to the JIS method.[23] The experimental results for total phosphorus were in good agreement with the JIS method.[23] Recovery of phosphorus added to the

TABLE 2. EFFECT OF FOREGIN IONS

Ions	Added as	Added ($\mu g/10\ cm^3$)	Added Mole ratio[a)]	P, Found ($\mu g/10\ cm^3$)	Recovery %
—	—	—	—	0.62	100.0
Fe(III)	Iron alum	5.6	5	1.14	183.9
		0.6	0.5	0.62	100.0
Al(III)	$AlCl_3$	2.7	5	1.02	164.5
		0.5	1	0.62	100.0
As(V)	Na_2HAsO_4	3.7	2.5	0.84	135.5
		1.5	1	0.62	100.0
Cu(II)	$Cu(NO_3)_2$	1.2	1	0.87	140.3
Zr(IV)	$ZrCl_4$	1.8	1	0.51	82.3
V(V)	NH_4VO_3	0.5	0.5	0.76	122.6
Mn(II)	$MnCl_2$	5.5	5	0.62	100.0
Co(II)	$Co(NO_3)_2$	5.9	5	0.62	100.0
Zn(II)	$ZnCl_2$	5.5	5	0.62	100.0
Pb(II)	$PbCl_2$	20.7	5	0.62	100.0
Ca(II)	$CaCl_2$	80.2	100	0.62	100.0
$C_2O_4^{2-}$	$Na_2C_2O_4$	17.6	10	0.54	87.1
Citrate	Sodium citrate	3.8	1	1.28	206.5
Tartrate	Sodium tartrate	3.0	1	0.86	138.7
SCN^-	NH_4SCN	11.6	10	0.51	82.3
NO_2^-	$NaNO_2$	92.0	100	0.62	100.0
CN^-	KCN	5.2	10	0.62	100.0
$S_2O_3^{2-}$	$Na_2S_2O_3$	22.4	10	0.62	100.0
F^-	NaF	76.0	200	0.62	100.0
I^-	KI	507.6	200	0.62	100.0
Cl^-	NaCl	141.8	200	0.62	100.0
NO_3^-	KNO_3	1240.1	1000	0.62	100.0
SO_4^{2-}	Na_2SO_4	1921.0	1000	0.62	100.0

P taken: 0.62 $\mu g/10\ cm^3$; Fe(III): 2.5×10^{-5} mol dm^{-3}; Qnph: 5.0×10^{-5} mol dm^{-3}; LT-221: 1.0 ml of 5.0% LT-221 solution/10 cm^3; Borax: 4.0×10^{-2} mol dm^{-3}; Reference: Solution B. a) Ion/Phosphorus.

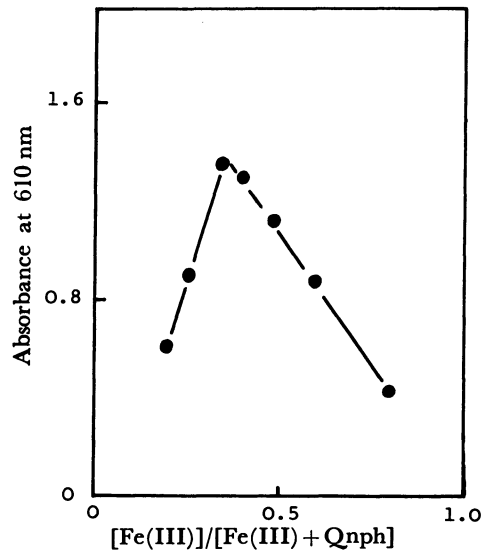

Fig. 4. Composition of [iron(III): Qnph] in the presence of phosphorus obtained by the continuous-variation method.
[iron(III)+Qnph]=5.0×10^{-5} mol dm^{-3}; phosphrus: 5.0×10^{-4} mol dm^{-3}; LT-221: 1.0 cm^3 of 5.0% LT-221 solution/10 cm^3; borax: 4.0×10^{-2} mol dm^{-3}; Reference: Solution B.

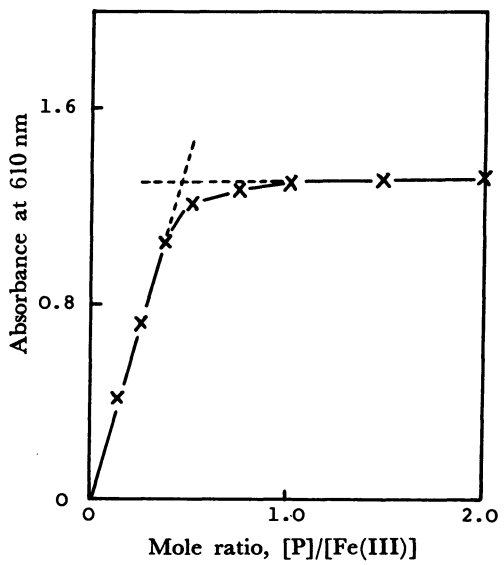

Fig. 5. Composition of [iron(III): Qnph] obtained by the molar-ratio method.
Iron(III): 2.0×10^{-5} mol dm^{-3}; Qnph: 5.0×10^{-5} mol dm^{-3}; phosphorus: 1.0×10^{-4} mol $dm^{-3}\times X$ $cm^3/10$ cm^3; LT-221: 1.0 cm^3 of 5% LT-221 solution/10 cm^3; borax: 4.0×10^{-2} mol dm^{-3}; Reference: Solution B.

TALE 3. ANALYTICAL RESULTS OF PHOSPHORUS IN WATER

Samples	Methods[a]	P, Found[b] (ppm)		Recovery % of the proposed method[c]/%
		Proposed method	JIS method	
A	(1)[a]	0.54	0.15	96.8
	(2)[a]	1.04	0.98	94.6
B	(1)[a]	0.23	0.17	104.3
	(2)[a]	0.25	0.22	96.0

Sample A, effluent of treated waste water supplied from the Center for Conservation, Osaka College of Pharmacy (April 17, 1984); Sample B, rain water on the campus of Osaka College of Pharmacy (April 19, 1984). a) Method (1), Phosphate ion was determined according to the JIS method; a) Method (2), Total phosphorus was determined according to the JIS method. b) Mean of 5 determinations. c) P taken, 0.31 μg/10 cm³; mean of 5 determinations.

TABLE 4. APPARENT MOLAR ABSORPTIVITIES FOR VARIOUS SUBSTANCES CONTAINING PHOSPHORUS BY THE STANDARD PROCEDURE

Substances	ε/dm³ mol⁻¹ cm⁻¹
PO_4^{3-}	2.5×10^5
$P_2O_7^{4-}$	1.7×10^5
$P_3O_{10}^{5-}$	2.0×10^5
5′-Adenine triphosphate	2.3×10^5
Flavin-adenine dinucleotide	8.0×10^4
β-Nicotinamide-adenine dinucleotide	1.9×10^4
D-Glucose 6-phosphate	4.2×10^4
Inosine 5′-phosphate	1.4×10^5
Triphenyl phosphate	2.5×10^3

Fe(III): 2.5×10^{-5} mol dm⁻³; Qnph: 5.0×10^{-5} mol dm⁻³; LT-221: 1.0 cm³ of 5.0% LT-221 solution/10 cm³; Borax: 4.0×10^{-2} mol dm⁻³; Reference: Solution B.

samples was satisfactory, about 95%. The results are given in Table 3.

Application to Other Compounds Containing Phosphorus. By using the standard procedure for the determination of phosphorus, other compounds containing phosphorus were measured without a preliminary treatment. Approximate apparent molar absorptivities of these compounds were calculated from the absorbance of Qnph–iron(III)-compounds containing phosphorus solutions at 610 nm against Solution B. The results are given in Table 4.

Conclusion

The fundamental conditions for the determination of phosphorus based on the ternary complex-formation with Qnph, an anionic dye, and iron(III) were discussed, and a new spectrophotometric method for micro amounts of phosphorus has been established. This method is applicable in the concentration range of ≈1 μg phosphorus in the final volume of 10 cm³. The apparent molar absorptivity in this procedure was estimated to be 2.5×10^5 dm³ mol⁻¹ cm⁻¹ for phosphorus. In sensitivity, this method is comparable to the EV-extraction method[9] or MG-extraction method.[13] The present method is aplicable to the determination of total phosphorus in samples containing micro amounts of metal ions, and an application to the determination of organic compounds containing phosphorus without a suitable preliminary treatment may also be feasible.

Though further investigation is necessary, the method proposed here gives an important suggestion for determining phosphorus by using an anionic dye.

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