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A Color Reaction of 1,2-Diphenols Based on Colored Complex Formation with Phenylfluorone and Iron(III) and Its Application to the Assay of Catecholamines in Pharmaceutical Preparations[1]

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A color reaction of 1,2-diphenols, such as catecholamines (CA), caffeic acid and gocypol, utilizing colored complex formation among a 1,2-diphenol, an organic reagent and a metal ion was studied, and suitable conditions for the spectrophotometric determination of 1,2-diphenols [as norepinephrine (NE), a CA] with phenylfluorone and iron(III) were established. The optimum pH range for the formation of colored complexes in the presence of polyoxyethylene monolauryl ether was pH 8.9—9.9. In the case of NE, the method could be used to determine up to *ca.* 10 μg/10 ml, and the apparent molar absorptivity at 630 nm was $1.7 \times 10^5\,\mathrm{dm^3\,mol^{-1}\,cm^{-1}}$. This method was applied to the assay of CA in commercial pharmaceutical preparations. A detection test for CA on a spot plate was also examined.

Keywords——1,2-diphenol; color reaction; spectrophotometry; catecholamine; norepinephrine; phenylfluorone; iron(III)

Various compounds[2-5] (such as caffeic acid, chlorogenic acid and esculetin) containing a 1,2-diphenolic nucleus in the molecule, including catecholamines (CA), are biologically and clinically important substances. Although numerous methods[6-10] for the determination of CA have been reported, little work has been done on methods for the determination of compounds containing a 1,2-diphenol nucleus. There are some methods,[11,12] for example the utilization of chelation with boric acid,[13-15] for the determination and detection of 1,2-diphenols, but these methods are not sufficiently sensitive and selective. Recently, Abd El Wahed *et al.*[16] determined the formation constants of chelates of dopamine and methyldopa with copper(II), iron(III) and zirconium(IV) in aqueous medium, and found that the chelation with iron(III) was the most stable.

Meanwhile, we have often reported that, in the determination of various organic compounds,[17] the use of both of an organic reagent and a metal ion offers advantages such as sensitivity, selectivity and simplicity as compared with the use of an organic reagent or a metal ion alone. A similar phenomenon was observed in the reaction system of a 1,2-diphenol, iron(III) and phenylfluorone (Phfl), a xanthene dye.

In this paper, a color reaction of 1,2-diphenols utilizing colored complex formation with a metal ion and an organic reagent was studied, and suitable conditions for the spectrophotometric determination of 1,2-diphenols were established. The proposed method was applied to the assay of CA in commercial pharmaceutical preparations. A detection test for CA on a spot plate was also examined.

Experimental

Reagents and Materials——CA and 1,2-Diphenols Solutions: Stock solutions (1.0×10^{-3} M, M = mol dm^{-3}) of

CA and 1,2-diphenols were prepared as aqueous or methanol solutions, and working solutions were made by dilution of these stock solutions as required. These solutions were stored in a cool, dark place. The CA and 1,2-diphenols used were norepinephrine hydrochloride (NE), epinephrine, isoproterenol hydrochloride, dopamine, dopa, methyldopa, catechol, caffeic acid, esculetin, apomorphine, chlorogenic acid, gocypol and tiron, purchased from Sigma Chemical Co., Tokyo Kasei Kogyo Co., Ltd., Dojindo Lab. and Nakarai Chemicals Ltd.

Organic Reagent Solutions: All reagent solutions were prepared in 1.0×10^{-3} M methanol solutions or methanol solutions containing one drop of hydrochloric acid. Organic reagents used were Phfl, *o*-hydroxyhydroquinonephthalein (Qnph), pyrogallol red (PR), gallein (Gall), pyrocatechol violet (PV), chromazurol S (CAS), 4-(2-pyridylazo)resorcinol (PAR), arsenazo III, tiron, 1,10-phenanthroline (Phen), alizarin S and chromotropic acid.

Iron(III) and Other Metal Ion Solutions: A solution of Iron(III) (1.0×10^{-3} M) was prepared by dissolving a suitable quantity of iron(III) ammonium sulfate in 5 ml (ml = cm^3) of 1.0% sulfuric acid and diluting the mixture to 100 ml with water. Solutions of other metal ions were prepared by dissolving suitable quantities of standard metal ions in the requisite volume of nitric acid or hydrochloric acid, or dissolving metal nitrates and metal chlorides in water.

Polyoxyethelene Monolauryl Ether (Brij-35) Solution: A 5.0% Brij-35 solution was prepared in water.

Buffer Solution: A 0.1 M sodium carbonate–0.1 M sodium hydrogen carbonate buffer solution was used for the pH adjustments.

All other chemicals were of analytical-reagent grade. Twice-distilled water was used throughout this work.

Apparatus——The absorption spectra and values of absorbance were measured using a Shimadzu UV-240 recording spectrophotometer with 1.0-cm slica cells. The values of pH were measured with a Hitachi-Horiba F-7 AD glass electrode pH meter.

Standard Procedure for the Spectrophotometric Assay of CA——The following components were added to a 10-ml calibrated flask; a sample solution containing up to *ca.* 10 μg of CA, 1.0 ml of 5.0% Brij-35 solution, and 0.25 ml of 1.0×10^{-3} M iron(III) solution. The solution was adjusted to *ca.* pH 9.5 with 3.0 ml of the buffer solution, then 0.25 ml of 1.0×10^{-3} M Phfl solution was added. The mixture was diluted to 10 ml with water and kept at room temperature for 30 min. The absorbance of the reaction mixture solution was measured at 630 nm against the Phfl–iron(III) solution.

Procedure for the Detection of CA on a Spot Plate——The following components were mixed in the cavity of a spot plate; 0.02 ml of the sample solution, 0.02 ml of 5.0% Brij-35 solution, 0.02 ml of 2.0×10^{-4} M iron(III) solution, 0.1 ml of the buffer solution and 0.02 ml of 1.0×10^{-3} M Phfl solution. The solution was mixed well with a glass rod. After few minutes, when CA was present in the sample solution, a positive response was indicated by the color change of the mixture from pale red to reddish-violet or violet.

Results and Discussion

Color Reaction among 1,2-Diphenol, Metal Ion and Organic Reagent, and Absorption Spectra

NE, which is a representative CA, was chosen for all further work. On the addition of NE to the Phfl–iron(III) solution, an absorbance maximum of the NE–Phfl–iron(III) solution at around 630 nm was distinctly observed; the absorbance was proportional to the concentration of NE. Similar phenomena were observed when other CA or 1,2-diphenols such as epinephrine, isoproterenol, catechol and caffeic acid were used instead of NE. The iron(III)–NE solution showed almost no absorption under the same conditions.

The effect of organic reagents was studied by measuring the difference of absorbance between NE–organic reagent–iron(III) and organic reagent–iron (III) solutions. As shown in Table I, Phfl, a xanthene dye, was superior to the other organic reagents tested in terms of sensitivity.

Next, the effect of metal ions was examined. Iron(III) and iron(II) were effective among various metal ions tested: iron(III), iron(II), cobalt(II), copper(II), manganese(II), aluminum(III), cerium(IV), molybdenum(VI), titanium(IV), *etc.* The results are given in Table II.

In this colored complex formation system, color reactions between the Phfl–iron(III) and various phenols or analogous compounds solutions were investigated. The results showed that the 1,2-diphenolic function with free adjacent positions was essential for the formation of the colored complexes. The results are given in Table III.

TABLE I. Effect of Organic Reagents

Reagent	Absorbance difference a)	at λ_{max}
—	0.008	470
Phfl	0.430	630
Qnph	0.150	610
PR	0.052	600
Gall	0.058	585
PV	0.110	615
CAS	0.010	525
XO	0.023	500
PAR	0.110	715
Arsenazo III	0.010	570
Tiron	0.010	510
Phen	0.008	460
Alizarin S	0.008	510
Chromotropic acid	0.008	500

NE, 2.5×10^{-6} M; Fe(III), 2.5×10^{-5} M; reagent, 2.5×10^{-5} M; Brij-35, 1.0 ml of 5.0% Brij-35 solution/10 ml; pH 9.5; reference, water. *a)* Difference of absorbance between NE–reagent–Fe(III) and reagent–Fe(III) solutions.

TABLE II. Effect of Metal Ions

Metal ion	Absorbance difference a)	at λ_{max}
—	0	—
Fe(III)	0.430	630
Fe(II)	0.350	630
Co(II)	0.100	520
Cu(II)	0.050	570
Mn(II)	0.092	560
Zn(II)	0.016	510
Pd(II)	0.026	545
Al(III)	0.028	525
Bi(III)	0.040	520
Ge(IV)	0.030	560
Ce(IV)	0.020	580
Ti(IV)	0.162	580
V(V)	0.030	540
Mo(VI)	0	—
Os(VIII)	0.038	555
$Fe(CN)_6^{3-}$	0.020	565

NE, 2.5×10^{-6} M; Phfl, 2.5×10^{-5} M; metal ion, 2.5×10^{-5} M; Brij-35, 1.0 ml of 5.0% Brij-35 solution/10 ml; pH 9.5; reference, water. *a)* Difference of absorbance between Phfl–metal ion–NE and Phfl–metal ion solutions.

The absorption spectra of NE–Phfl–iron(III), Phfl–iron(III) and Phfl solutions under the standard conditions are shown in Fig. 1.

Effect of pH

Maximum and almost constant absorbance of the NE–Phfl–iron(III) solution was observed in the pH range of 8.9—9.9 when the final solution was adjusted with 3.0 ml of 0.1 M sodium carbonate–0.1 M sodium hydrogen carbonate buffer solution.

TABLE III. Reaction between Various Phenols or Analogous Compounds and Phfl–Fe(III) Solution

Compound	Phenol classification	Added (M)	Absorbance at 630 nm
NE	1,2-Diphenol	2.5×10^{-6}	0.430
Catechol	1,2-Diphenol	2.5×10^{-6}	0.410
Pyrogallol	1,2,3-Triphenol	1.0×10^{-5}	0.365
Phloroglucinol	1,3,5-Triphenol	5.0×10^{-4}	0.150
Hydroquinone	1,4-Diphenol	5.0×10^{-4}	0.040
Resorcinol	1,3-Diphenol	5.0×10^{-4}	−0.030
Phenol	Monophenol	5.0×10^{-4}	−0.020
Caffeic acid	1,2-Diphenol	2.5×10^{-6}	0.230
Esculetin	1,2-Diphenol	2.5×10^{-6}	0.154
Octopamine	Monophenol	5.0×10^{-4}	−0.020
Norephedrine	—	5.0×10^{-4}	−0.250
Phenylalanine	—	5.0×10^{-4}	−0.070
Guaiacol	Monophenol	5.0×10^{-4}	−0.070
2,3-Dihydroxybenzoic acid	1,2-Diphenol	2.5×10^{-6}	0.135
3,5-Dihydroxybenzoic acid	1,3-Diphenol	5.0×10^{-4}	−0.042
Anthranilic acid	—	5.0×10^{-4}	0
Salicylic acid	—	5.0×10^{-5}	−0.205
Phen	—	5.0×10^{-5}	−0.315

Fe(III), 2.5×10^{-5} M; Phfl, 2.5×10^{-5} M; Brij-35, 1.0 ml of 5.0% Brij-35 solution/10 ml; pH 9.5; reference, the Phfl–Fe(III) solution.

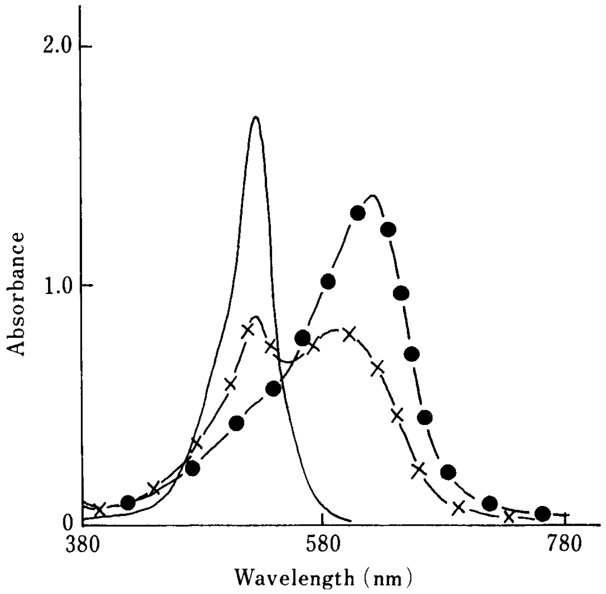

Fig. 1. Absorption Spectra of NE–Phfl–Fe(III), Phfl–Fe(III) and Phfl Solutions under the Standard Conditions

NE, 5.0×10^{-6} M; Fe(III), 2.5×10^{-5} M; Phfl, 2.5×10^{-5} M; Brij-35, 1.0 ml of 5.0% Brij 35 solution/10 ml; pH 9.5; reference, water.
—●—, NE–Phfl–Fe(III); —×—, Phfl–Fe(III); ——, Phfl.

Effect of Surfactants

Among various surfactants examined, Brij-35, a nonionic surfactant, was most effective with respect to sensitivity; maximum and constant absorbance was obtained with more than 0.5 ml of 5.0% Brij-35 solution in the final volume of 10 ml.

Effect of Iron(III) and Phfl Concentrations

The effects of the amounts of iron(III) and Phfl were examined by varying the molar ratio of Phfl to iron(III), the amount of NE being kept constant. Though the molar ratio of iron(III) to Phfl in the complex was found to be 2 : 3 in the presence of NE by the continuous-

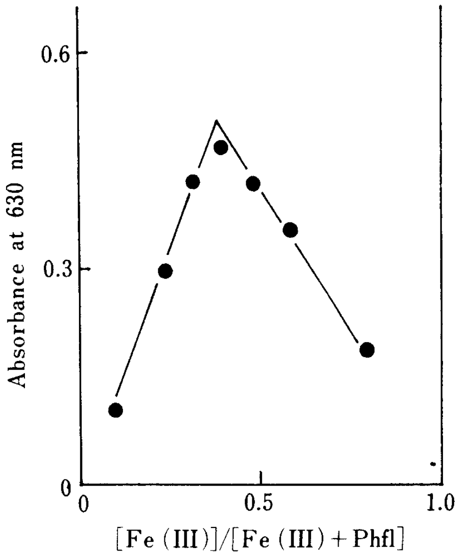

Fig. 2. Composition of [Fe(III) : Phfl] in the Presence of NE, Obtained by the Continuous-variation Method

[Fe(III) + Phfl] = 2.0×10^{-5} M; NE, 5.0×10^{-5} M; Brij-35, 1.0 ml of 5.0% Brij-35 solution/10 ml; pH 9.5; reference, Phfl solution.

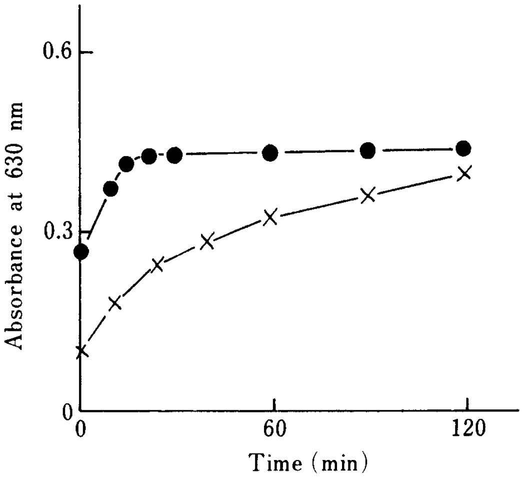

Fig. 3. Relationship between Standing Time and Absorbance at a Molar Ratio of Fe(III) 1 : Phfl 1 or Fe(III) 2 : Phfl 3 at Room Temperature

NE, 2.5×10^{-6} M; Fe(III), 2.5×10^{-5} M; Brij-35, 1.0 ml of 5.0% Brij-35 solution/10 ml; pH 9.5; reference, the Phfl–Fe(III) solution.
—●—, [Fe(III) 1 : Phfl 1]; —×—, [Fe(III) 2 : Phfl 3].

Table IV. The Apparent Molar Absorptivities of CA and Some 1,2-Diphenols Obtained by the Present Method

Compound	$\varepsilon^{a)}$ ($dm^3 mol^{-1} cm^{-1}$)
NE	1.7×10^5
Epinephrine	1.6×10^5
Isoproterenol	1.4×10^5
Dopamine	1.3×10^5
Dopa	1.3×10^5
Methyldopa	1.1×10^5
Catechol	1.6×10^5
Caffeic acid	9.0×10^4
Esculetin	6.0×10^4
Apomorphine	9.0×10^4
Chlorogenic acid	1.1×10^5
Gocypol	4.0×10^4
Tiron	2.9×10^4

Fe(III), 2.5×10^{-5} M; Phfl, 2.5×10^{-5} M; Brij-35, 1.0 ml of 5.0% Brij-35 solution/10 ml; pH 9.5; reference, the Phfl–Fe(III) solution. *a*) Apparent molar absorptivity at 630 nm.

variation method, the use of [iron(III) 1 : Phfl 1] was superior to the use of [iron(III) 2 : Phfl 3] in terms of reactivity and stability at room temperature. The results are shown in Figs. 2 and 3.

Accordingly, all further work was carried out with 2.5×10^{-5} M iron(III) and 2.5×10^{-5} M Phfl in the final volume of 10 ml, which resulted in an appropriate determination limit for CA.

Calibration Curves for CA and Some 1,2-Diphenols

Under the optimum conditions described above, a calibration curve for NE was

TABLE V. Effect of Foreign Substances

Substance	Added		NE found, μg/10 ml
	μg/10 ml	Mole ratio[a]	
—	—	—	4.2
Fe(III) (Alum)	1.4	1	4.9
Cu(II) (Nitrate)	3.1×10^{-1}	1/5	6.6
Co(II) (Nitrate)	7.4	5	4.2
Mg(II) (Chloride)	3.0	5	4.2
KH_2PO_4	6.8×10^{-1}	1/5	4.9
Na_2SO_3	6.3×10	20	3.9
NH_4SCN	1.9×10^2	100	4.2
H_3BO_3	3.1×10	20	4.2
Sodium citrate	6.5	1	5.6
L-Ascorbic acid	2.2	1/2	5.5
Pyridoxine	4.2×10	10	4.6
Salicylic acid	6.9×10	20	3.4
Chlorpheniramine	6.9×10^2	100	4.2
Ampicillin	8.7×10^2	100	4.7
Phenolpropanolamine	8.0×10	50	4.2
Caffeine	9.7×10^2	200	3.9
Saccharin	4.6×10^2	100	4.2
Taurine	6.3×10^2	200	4.2
D-Glucose	4.5×10^2	200	4.2
Urea	3.0×10^2	200	4.2
Creatinine	2.8×10^2	100	5.0
Oxine	3.6	1	1.8
Phenol	4.7×10^2	200	3.9
Resorcinol	5.5×10^2	200	4.0
Octopamine	7.7×10^2	200	4.0
Albumin	1.0×10^2	—	3.3
Sodium chondroitin sulfate	2.0×10^2	—	4.0

NE, 2.5×10^{-5} M; Fe(III), 2.5×10^{-5} M; Phfl, 2.5×10^{-5} M; Brij-35, 1.0 ml of 5.0% Brij-35 solution/10 ml; pH 9.5; reference, the Phfl–Fe(III) solution. *a*) Substance/NE.

constructed based on absorbance at 630 nm. A good linear relationship was observed over the range up to 10 μg of NE in the final volume of 10 ml. The effective molar absorptivity for NE was calculated to be 1.7×10^5 $dm^3 mol^{-1} cm^{-1}$. When the determination of a solution containing 4.2 μg of NE was repeated 6 times by the standard procedure, the coefficient of variation was 1.5%. Other CA were also determined in quantities up to *ca.* 10 μg/10 ml. Table IV lists the apparent molar absorptivities of CA and some 1,2-diphenols at 630 nm.

Effect of Foreign Substances

The interferences by various ions and substances in the determination of 4.2 μg of NE were studied. Among metal ions examined, copper(II) and iron(III) interfered with the determination, but other metal ions such as cobalt(II), zinc(II), manganese(II), magnesium(II), *etc.* did not interfere even when present in 5-fold excess over NE. Among the anions examined, phosphate and organic acid ions such as citrate gave small positive errors. Though salicylic acid, 8-hydroxyquinoline (Oxine), albumin and chondroitin sulfate gave negative errors, phenylpropanolamine, octopamine, chlorpheniramine, D-glucose, urea, taurine, *etc.* did not interfere when present in 50- to 200-fold excess over NE. The results are summarized in Table V.

TABLE VI. Determination of CA in Commercial Pharmaceutical Preparations

Sample	Amount (mg)		Recovery test[a]	
	Nominal	Found[a]	Added (μg/10 ml)	%
A	1.0	1.06	2.5	98.1
B	25.0	25.3	5.9	95.4
C	7.5	7.1	5.0	99.2
D	250.0	243.0	7.1	102.2

Sample A, NE injection (in 1 ml); sample B, dopa injection (in 10 ml); sample C, isoproterenol capsule (in 1 cap., 1 cap. ≒ 150 mg); sample D, methyldopa tablet (in 1 tab., 1 tab. ≒ 350 mg). *a*) Average of 5 determinations.

Composition of the Complex

As mentioned above, the molar ratio of iron(III) to Phfl was 2 : 3 in the presence of NE. The NE-to-iron(III) ratio obtained by the molar-ratio method was 1 : 1. On the other hand, the molar ratio of iron(III) to Phfl was 3 : 2 in the absence of NE. Though further investigation is necessary, the colored complex formed in this reaction system appears to be a mixed ligand complex which can be expressed as $[(NE)_2(Fe^{III})_2 (Phfl)_3]$.

Application to the Assay of CA in Pharmaceutical Preparations

The spectrophotometric procedure was applied to the assay of CA in commercial pharmaceutical preparations. The samples examined were NE injection, dopa injection, isoproterenol capsule and methyldopa tablet. The recoveries of CA added to the samples were satisfactory, being about 95–102%. The results are given in Table VI.

Detection Test for CA on a Spot Plate

A detection test for CA on a spot plate using Phfl and iron(III) was also investigated in the previous report.[17b,c] The less the amount of iron(III), the clearer was the color contrast between the sample and blank; the molar ratio of Phfl to iron(III) should be greater than 2.0.

Thus, satisfactory results were obtained when detection tests were carried out according to the procedure described in Experimental. The detection limit for CA on a spot plate was approximately 0.05 μg in 0.02 ml of the sample solution.

Conclusion

A color reaction of 1,2-diphenols based on colored complex formation among a 1,2-diphenol, an organic reagent and a metal ion has been developed, and a spectrophotometric assay of 1,2-diphenols (as CA) with Phfl and iron(III) has been established. This method could be used in the range of up to about 10 μg of CA. In the determination of NE, the apparent molar absorptivity at 630 nm was 1.7×10^5 dm^3 mol^{-1} cm^{-1}, and this procedure was about twice as sensitive as the method[18] using 2,3,5-triphenyltetrazolium. Our method was applied to the assay of CA in pharmaceutical preparations, and the results were satisfactory. The detection test on a spot plate was also effective. The proposed method seems to be suitable for use as a simple and sensitive assay for various compounds containing a 1,2-diphenolic function, including CA.

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1) This paper is Part XLVII of a series entitled "Application of Xanthene Derivatives for Analytical Chemistry,"

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