Spectrophotometric Determination of Ascorbic Acid with Iron(III) and *p*-Carboxyphenylfluorone in a Cationic Surfactant Micellar Medium

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A simple and highly sensitive spectrophotometric method for the determination of ascorbic acid (AA) was established by using iron(III) and *p*-carboxyphenylfluorone (PCPF) in a cationic surfactant micellar medium. The apparent molar absorptivity of the proposed method, which does not require an extraction procedure, was $2.05 \times 10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 655 nm. Beer's law was obeyed in the concentration range of 0.02–0.12 $\mu\text{g}/\text{cm}^3$ for AA. The procedure was successfully applied to assays of AA in pharmaceutical preparations. It is suggested that the method is based on a coupled redox-complexation reaction in which the first step is the oxidation of AA by iron(III), and the second step includes the formations of the iron(II)-PCPF (1:2) complex and the dehydroascorbic acid-iron(III)-PCPF (1:1:2) complex.

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Ascorbic acid (AA)¹ is an important vitamin which participates in a great variety of biological events concerning electron-transport reactions, hydroxylations, the oxidative catabolism of aromatic amino acids and so on. Numerous papers and reviews²⁻⁵ have described the determination of AA. The methods used include titrimetric, spectrophotometric, fluorometric, electrochemical, chromatographic, kinetic and chemiluminescent procedures. Photometric methods continue to be of interest because of ease in accessibility and their quick applicability to routine analyses. The majority of these methods are based on their oxidation-reduction properties or ability to couple with diazotized aniline derivatives. Many spectrophotometric methods based on the reduction of iron(III) to iron(II) with AA, followed by complexation of reduced iron(II) with different reagents, such as 1,10-phenanthroline (phen) and 2,2'-dipyridyl, have been reported. Another fast and facile method based on a proportional decrease in the absorbance of iron(III) complexes by the addition of AA has been proposed by some authors. On the other hand, AA has several donor atoms capable of metal complex formation and binding^{6,7} with zinc(II), manganese(II), cadmium(II), alkaline earth metals and so on.

We have already reported⁸⁻¹⁰ on simple and sensitive determinations of various organic compounds utilizing a mixed-complex system among an organic compound, a metal ion and a dye. Here, we have studied the color reaction of AA based on a coupled redox-complexation reaction among iron(III), xanthene dye and AA; also, a simple and highly sensitive spectrophotometric determination of AA was investigated by using iron(III) and *p*-carboxyphenylfluorone (PCPF) as a dye in the presence of a cationic surfactant. The proposed method was

then applied to assays in pharmaceutical preparations.

Experimental

Reagents and solutions

AA and dehydroascorbic acid (DHAA), analytical-grade reagents, were purchased from Wako Pure Chemical Co. Ltd., and used without further purification. The stock and working solutions of AA were freshly prepared every day. DHAA was prepared daily not more than 2 h prior to use by dissolving DHAA in water. A solution of iron(III) ($5.0 \times 10^{-4} \text{ M}$, $1 \text{ M} = 1 \text{ mol dm}^{-3}$) was prepared by dissolving a calculated amount of ferric ammonium sulfate in 100 cm^3 of water containing 1 cm^3 of hydrochloric acid (HCl) and a 10-fold molar excess of sodium fluoride over iron(III). A $1.0 \times 10^{-3} \text{ M}$ solution of PCPF, which had been synthesized according to a method described in the literature,^{11,12} was prepared in methanol containing one drop of HCl. A $1.0 \times 10^{-2} \text{ M}$ solution of *n*-tetradecyltrimethylammonium chloride (TTAC) was prepared in water. A buffer solution of pH 8.5 was prepared by mixing 0.1 M HCl and 0.1 M 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) solutions. All other reagents and materials were of analytical grade, and were used without further purification. Pure water was prepared by purifying deionized water with a Milli-Q Labo System just before use.

Apparatus

A Shimadzu (Model UV-160) recording spectrophotometer with 1.0-cm silica cells was used for conventional measurements. The pH measurements were made with a Horiba (Model F-11) pH meter in combination with a calomel glass electrode.

Standard procedure for the determination of AA

The following components were mixed in a 10- cm^3

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Table 1 Effect of dyes

Dye	Absorbance at $\lambda_{\max}$	
PCPF	0.502	655
SPF	0.389	643
PF	0.317	625
DSPF	0.302	650
QP	0.248	635
SF	0.213	623

[AA] = 4.0×10^{-7} M; [Fe(III)] = 1.5×10^{-5} M; [F⁻] = 1.5×10^{-3} M; [Dye] = 6.0×10^{-5} M; [TTAC] = 2.5×10^{-3} M; pH, 8.5; reference, reagent blank.

volumetric flask: a solution containing from 0.2 to 1.2 μ g of AA, 0.3 cm³ of a 5.0×10^{-4} M iron(III) solution, 2.5 cm³ of a 1.0×10^{-2} M TTAC solution, 2.5 cm³ of the buffer solution and 0.6 cm³ of a 1.0×10^{-3} M PCPF solution. The mixture was then made up to volume with water, transferred into a test tube, mixed well and kept at 60°C for 20 min. After the solution had been cooled for 5 min in water, the absorbance of the resultant solution (Solution A) was measured at 655 nm against a reagent blank (Solution B) prepared in the same way.

Results and Discussion

Preliminary experiments and choice of dye

We preliminarily examined the utility of a method for determining AA by comparing with the color reactions between iron(III) and several xanthene dyes in the presence of AA at various pH media (*ca.* pH 4–10). An AA-iron(III)-dye solution in the presence of a cationic surfactant exhibited a considerable red shift compared to an iron(III)-dye solution in a moderate basic medium; the color reaction was found to be highly sensitive. Because an iron(III)-dye-AA solution without a surfactant formed a precipitate under specific conditions, the effects of different surfactants were examined as a dispersion agent. The coexistence of a cationic surfactant was superior to that of a non-ionic, anionic or amphoteric surfactant regarding the sensitivity and color contrast between the sample and the reagent blank solutions. The xanthene dyes examined were PCPF, *o*-hydroxyhydroquinonephthalein (QP), *o*-sulphophenylfluorone (SPF), phenylfluorone (PF), salicylfluorone (SF) and 2,4-disulphophenylfluorone (DSPF). The structures and sensitivities of these dyes are given in Table 1 and Fig. 1.

Optimization of the conditions and absorption spectra

Of the various buffer solutions tested, the maximum and constant absorbance value was obtained in the pH range of 8.3 to 8.7 with 0.1 M Tris and 0.1 M HCl buffer solutions.

In order to stabilize the colored complex formed and to increase the sensitivity, the effect of the coexistence of several cationic surfactants was examined in the concentration range from 1.0×10^{-4} M to 2.0×10^{-2} M. The structures, optimal concentrations, critical micelle concentrations (CMC) and sensitivity of the surfactants examined are given in Table 2. These CMC were spectrophotometrically determined based on the following procedure. In a 10-cm³ volumetric flask were mixed a solution containing a series of concentrations of each cationic surfactant, 2.5 cm³ of the buffer solution and 0.6 cm³ of a 1.0×10^{-3} M PCPF solution. The mixture was then made up to volume with water, transferred into a test tube, mixed well and kept at 60°C for 20 min. After the solution had cooled for 5

Fig. 1 Structures of the dyes studied.

Dye	R ₁	R ₂
PCPF	H	COOH
QP	COOH	H
SPF	SO ₃ H	H
PF	H	H
SF	OH	H
DSPF	SO ₃ H	SO ₃ H

min in water, the absorbance of the resultant solution was measured at 520 nm against a reagent blank without a cationic surfactant prepared in the same way. The optimal concentration of each cationic surfactant was observed in about a 4–8 fold molar excess over CMC. Higher concentrations of the optimal concentrations showed remarkably a decrease in the absorbance. In general, a higher absorbance of a dye-metal complex appears at a low concentration of a quaternary ammonium salt with higher order complexation, and becomes more difficult along with an increase of quaternary ammonium salt.^{13,14} From Table 2, an iron(III)-dye-AA solution in the presence of TTAC showed the greatest red shift of all, and the maximum and constant absorbance value was obtained by the addition of 2.2–2.6 cm³ of a 1.0×10^{-2} M TTAC solution in the final volume of 10 cm³.

The effects of the amounts of PCPF and iron(III) were examined. The results indicated that the molar ratio of PCPF to iron(III) should be greater than four-fold. The effect of the amount of fluoride ion was then examined, with the amount of iron(III) being kept constant. The maximum, constant-absorbance value was observed when fluoride ion was added at about 8- to 12-fold molar excess over iron(III). Accordingly, all further work was carried out with 1.5×10^{-5} M iron(III), 1.5×10^{-4} M fluoride ion and 6.0×10^{-5} M PCPF solutions in the final volume of 10 cm³, while considering the absorbance of Solution B.

The effect of the sequence of adding of reagents on the absorbance values was examined. The maximum absorbance was obtained by keeping the AA solution first, and a subsequent addition of iron(III) solution.

The color development in this reaction system did not occur instantaneously at room temperature, more than 120 min being required for completion of the color reaction. The effects of the incubation temperature and times were examined by heating for 10–60 min at various temperatures (40°C, 50°C, 60°C and 70°C). An almost constant absorbance was obtained at 60°C for 20–30 min or at 70°C for 15–30 min, followed by cooling to room temperature. Solutions A and B upon heating at 70°C were apt to form aggregates; the reproducibility was somewhat poor. The absorbance of Solution A upon heating for 60 min at 40°C or 50°C did not reach the maximal value. The absorbance of Solution A against Solution B while being kept at 60°C for 20 min and then cooled for 5 min in water remained constant for at least 3 h.

Figure 2 shows the absorption spectra of Solutions A and B under the standard procedure. The absorption maximum of Solution B ($\lambda_{\max}$: 600 nm) gradually exhibited a red shift with increasing AA concentrations, and a distinct difference in the absorbance between Solution A and Solution B was observed at around 655 nm. The absorbance difference was proportional to the concentration of AA, and the absorbance of Solution B at 655 nm was relatively low.

Table 2 Structures, optimal concentrations and sensitivities of cationic surfactants studied

Cationic surfactant ^a	Structure	Optimal concentration/ M	Absorbance at $\lambda_{\max}$	Critical micelle concentration/M
Dodecyltrimethylammonium	$\text{CH}_3(\text{CH}_2)_{11}\text{N}(\text{CH}_3)_3$	$(1.0 - 1.6) \times 10^{-2}$	0.142(650)	— ^b
TTAC	$\text{CH}_3(\text{CH}_2)_{13}\text{N}(\text{CH}_3)_3$	$(2.2 - 2.6) \times 10^{-3}$	0.502(655)	4.5×10^{-4}
Cetyltrimethylammonium	$\text{CH}_3(\text{CH}_2)_{15}\text{N}(\text{CH}_3)_3$	$(6.5 - 8.0) \times 10^{-4}$	0.281(640)	1.7×10^{-4}
Stearyltrimethylammonium	$\text{CH}_3(\text{CH}_2)_{17}\text{N}(\text{CH}_3)_3$	$(2.5 - 4.5) \times 10^{-4}$	0.248(640)	5.3×10^{-5}
Cetylpyridinium	$\text{CH}_3(\text{CH}_2)_{15}\text{NC}_5\text{H}_5$	$(5.0 - 8.0) \times 10^{-4}$	0.282(640)	1.4×10^{-4}
Tetradecyldimethylbenzylammonium	$\text{CH}_3(\text{CH}_2)_{13}\text{N}(\text{CH}_3)_2\text{CH}_2\text{C}_6\text{H}_5$	$(1.4 - 1.8) \times 10^{-3}$	0.222(640)	2.8×10^{-4}
Cetyldimethylbenzylammonium	$\text{CH}_3(\text{CH}_2)_{15}\text{N}(\text{CH}_3)_2\text{CH}_2\text{C}_6\text{H}_5$	$(3.0 - 4.0) \times 10^{-4}$	0.192(640)	— ^b

a. All cationic surfactants were used chlorides. b. Undetermined.

Calibration curve

A calibration graph was constructed in the usual way according to the standard procedure. The linear range was obeyed for $0.02 - 0.12 \mu\text{g}/\text{cm}^3$ of AA. The apparent molar absorptivity (ϵ) was calculated from the slope of the calibration graph to be $2.05 \times 10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$. This procedure is much more sensitive than other spectrophotometric methods, using iron(III)-nitroso R¹⁵ ($\epsilon = 4.5 \times 10^4$), copper(II)-5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin¹⁶ ($\epsilon = 2.0 \times 10^4$), iron(III)-phen in the presence of cetylpyridinium bromide¹⁷ ($\epsilon = 2.6 \times 10^4$), copper(II)-EDTA complex¹⁸ ($\epsilon = 1.52 \times 10^4$), silver gelatin complex¹⁹ ($\epsilon = 2.15 \times 10^4$) and iron(III)-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol²⁰ ($\epsilon = 1.31 \times 10^4$), and is as sensitive as the Leuco Crystal Violet method ($\epsilon = 1.6 \times 10^6$).²¹ The relative standard deviation (RSD) for five runs of $0.7 \mu\text{g}$ AA was 2.2%. Though a shortcoming of this method is that the calibration curve does not pass through the origin, the proposed method has the following positive points: (1) higher sensitivity than conventional methods and (2) wider linear part in the calibration curve.

Interference of foreign substances

The influence of various foreign substances on the determination of AA was examined. Inorganic ions, such as ammonium, sodium, potassium, calcium, magnesium, chloride, bromide, nitrate, sulfate, and acetate ions, did not noticeably affect the accuracy of the determination of AA, even when these ions were present in large excess amounts compared with that of AA. Sulfite and thiosulfate ions caused minor errors in one thousand-fold excess over AA. Nitrite and iodide ions interfered severely in small amounts. A fairly large error appeared in the presence of oxalate, citrate ions and hydroxylamine. The presence of other vitamins, such as pyridoxine, thiamine, riboflavin, nicotinamide, panthothenate and cyanocobalamin, interfered very little. Two-hundred-fold amounts of caffeine, salicylate, glycine, glucose, lactose, urea and creatinine did not interfere with the determination of AA. The results are summarized in Table 3.

Application

The proposed method was applied to the determination of AA in commercial pharmaceutical preparations (powder, granule and tablet). The contents of powder, granules or tablets were accurately weighed and ground in a mortar to a fine powder. The required amount of powder was weighed, transferred into a 100-cm^3 volumetric flask, diluted to the mark with water and filtered. An appropriate amount of the sample solution was taken and assayed according to the standard procedure. The results of applying the proposed method showed good agreement with those obtained by the Japanese Pharmacopoeial

Fig. 2 Absorption spectra of AA-Fe(III)-PCPF (Solution A) and Fe(III)-PCPF (Solution B) solutions obtained under the standard procedure. [AA] = 4.0×10^{-7} M (Curve 1), 8.0×10^{-7} M (Curve 2); [Fe(III)] = 1.5×10^{-5} M; [F⁻] = 1.5×10^{-3} M; [PCPF] = 6.0×10^{-5} M; [TTAC] = 2.5×10^{-3} M; pH, 8.5; Curves 1 and 2, Solution A, Curve 2, Solution B, reference, water.

method.²² The accuracy of the proposed method was checked by a thorough replicate analysis of each sample spiked with $0.07 \mu\text{g}/\text{cm}^3$ of AA. Neat standards were prepared under the same conditions. The recovery was determined by comparing the standard absorbance. The recoveries of the added quantity were about 97 – 103%. This indicates that the proposed method gives accurate results. These results are given in Table 4.

Composition of the colored complex

In order to clarify the reaction mechanism, the compositions of the colored complexes were studied by Job's method of continuous variation and the molar ratio method. AA is readily oxidized to DHAA by iron(III), and iron(III) ion is reduced to iron(II). Thus, iron(II), iron(III), DHAA and PCPF are supposed to coexist in Solution A. The iron(III)-to-PCPF ($\lambda_{\max}$: 600 nm, Fig. 3-(a)) ratio and the iron(II)-to-PCPF ($\lambda_{\max}$: 640 nm, Fig. 3-(b)) were 1:1 and 1:2 in the absence of AA, respectively. The reaction rate between iron(II) and PCPF was much faster than that between iron(III) and PCPF. The iron(III)-to-PCPF ratio ($\lambda_{\max}$: 640 nm, Fig. 3-(c)) was found to be 1:2 in the presence of DHAA. Furthermore, the iron(III)-to-DHAA (Fig. 3-(d)) ratio was found to be 1:1, whereas the binding of iron(II) with AA or DHAA could not be observed. The results are

Table 3 Effect of foreign substances on assay of AA

Substance	Added (Substance/AA)	Absorbance at 655 nm	Recovery, %
NaCl	1.0×10^3	0.502	100.0
K ₂ SO ₄	1.0×10^3	0.502	100.0
CH ₃ COONa	1.0×10^3	0.502	100.0
NH ₄ Cl	1.0×10^3	0.502	100.0
NaNO ₃	1.0×10^3	0.502	100.0
KBr	1.0×10^3	0.502	100.0
Mg(II), chloride	1.0×10^3	0.502	100.0
Ca(II), chloride	1.0×10^3	0.502	100.0
NaI	1.0×10	0.445	88.6
NaNO ₂	2.5×10^2	0.413	82.2
Na ₂ SO ₃	1.0×10^3	0.401	80.0
Na ₂ S ₂ O ₃	1.0×10^3	0.446	88.8
NH ₂ OH·HCl	1.0	0.567	113.0
Sodium oxalate	1.0	0.580	115.5
Sodium citrate	5.0	0.572	113.9
Pyridoxine·HCl	2.0×10	0.502	100.0
Thiamine·HCl	2.0×10	0.502	100.0
Riboflavin	2.0×10	0.502	100.0
Nicotinamide	2.0×10	0.502	100.0
Panthothenate	2.0×10	0.502	100.0
Cyanocobalamin	2.0×10	0.502	100.0
Caffeine	2.0×10^2	0.502	100.0
Sodium salicylate	2.0×10^2	0.502	100.0
Glycine	2.0×10^2	0.502	100.0
Glucose	2.0×10^2	0.502	100.0
Lactose	2.0×10^2	0.502	100.0
Urea	2.0×10^2	0.502	100.0
Creatinine	2.0×10^2	0.502	100.0

[AA] = 4.0×10^{-7} M; [Fe(III)] = 1.5×10^{-5} M; [F⁻] = 1.5×10^{-3} M; [PCPF] = 6.0×10^{-5} M; [TTAC] = 2.5×10^{-3} M; pH, 8.5; reference, reagent blank.

shown in Fig. 3.

The experimental results imply that the iron(III) ion reacted with AA to give the iron(II) ion in the first step, followed by the formation of the iron(II)-PCPF (1:2) complex. At the same time, the residual iron(III) ion reacted with DHAA and PCPF to give the DHAA-iron(III)-PCPF (1:1:2) complex. Accordingly, the reaction mechanism in this reaction system is assumed to take place according to the following schema:

However, the purity of commercially available DHAA has been questioned,²³ and DDHA is further oxidized to 2,3-diketogulonic acid. In addition, the binding of TTAC could not be determined. There is room for further investigation by using other methods, such as ESR and NMR spectroscopies.

Conclusion

A simple and highly sensitive spectrophotometric method for AA was established with iron(III) and PCPF in a cationic surfactant micellar medium. The sensitivity ($\epsilon = 2.05 \times 10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) was much more sensitive than that using other spectrophotometric methods. The proposed method, owing to no need for solvent extraction, was applied to assays of AA in pharmaceutical preparations; the analytical results were satisfactory. It was suggested that the colored complex

Table 4 Determination of AA in pharmaceutical preparations

Sample	Content/mg ^{a,b}			RSD, %	Recovery, ^b %
	Nominal amount	Present method	Other method ^c		
Powder A	50	52.1	52.5	2.3	98.6
Granule A	250	256.2	255.8	2.7	96.5
	B	667	650.5	2.3	101.0
Tablet A	100	101.5	— ^d	2.8	103.4
	B	250	248.6	— ^d	97.2
	C	100	97.6	— ^d	103.5
	D	500	513.4	— ^d	102.2

a. Drug content, mg per 1 powder, mg per 1 granule or mg per 1 tablet.

b. Mean of 5 determinations.

c. The JP XIII method.

d. Undetermined.

Fig. 3 Composition of the complex obtained by the continuous variation method (a) - (c) and the molar ratio method (d). (a) [Fe^{III} + PCPF] = 1.0×10^{-5} M in the absence of AA, (b) [Fe^{II} + PCPF] = 1.0×10^{-5} M in the absence or presence of AA (1.0×10^{-4} M), (c) [Fe^{III} + PCPF] = 1.0×10^{-5} M in the presence of DHAA (1.0×10^{-4} M), (d) [Fe^{III}] = [PCPF] = 5.0×10^{-6} M, [DHAA] = 1.0×10^{-4} M $\times$ X ml, (a) - (d) [TTAC] = 2.5×10^{-3} M; pH, 8.5; reference, reagent blank.

produced was a mixture of the iron(II)-PCPF (1:2) complex and the DHAA-iron(III)-PCPF (1:2:2) complex. Though further investigations are necessary concerning the reaction mechanism and reaction species, the proposed method should be useful for a simple and highly sensitive determination of AA in real samples.

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