—Regular Article—

Improved Spectrophotometric Determination of Total Iron and Iron(III) with *o*-Hydroxyhydroquinonephthalein and Their Characterization

Mitsuru HOSHINO,[a] Hiroyuki YASUI,[b] Hiromu SAKURAI,[c] Takako YAMAGUCHI,[a] and Yoshikazu FUJITA*,[a]

[a]*Osaka University of Pharmaceutical Sciences, 4–20–1 Nasahara, Takatsuki, Osaka 569–1094, Japan,* [b]*Kyoto Pharmaceutical University, 5 Misasagi-Nakauchicho, Yamashina, Kyoto 607–8414, Japan, and* [c]*Suzuka University of Medical Science, 3500–3 Minami-tamagaki-cho, Suzuka, Mie 513–8670, Japan*

(Received March 23, 2011; Accepted April 19, 2011; Published online April 25, 2011)

Simultaneous and fractional determination of iron(II) and iron(III) was accomplished with *o*-hydroxyhydroquinonephthalein (QP) in the presence of poly (*N*-vinyl pyrrolidone). In the determination of total iron (iron(II) + iron(III)), Beer's law was obeyed in the range of 0.02–0.67 $\mu g \cdot ml^{-1}$, with an effective molar absorptivity (at 570 nm) and a relative standard deviation of $1.30 \times 10^5 \cdot l \cdot mol^{-1} \cdot cm^{-1}$ and 0.77% ($n=8$), respectively. This method was about 10–15 times and more than the methods using 1,10-phenanthroline and 2,2′-bipyridine. In addition, the iron-QP complex was characterized using spectrophotometry and the electron spin resonance. This method was successfully applied to assays of total iron and iron(III) in pharmaceutical preparations.

Key words——total iron; iron(III); spectrophotometry; *o*-hydroxyhydroquinonephthalein; characterization

INTRODUCTION

Iron[1] is an abundant element with a Clarke number of 4.70, the fourth largest among the elements, and it is an essential component of almost every organism in the biosphere. Because the standard oxidation-reduction potential of iron is at an intermediate level (+0.77 V), iron(III) and iron(II) always coexist. However, the behaviors of iron(III) and iron(II) and their environmental dynamics have not been clarified in detail, even though there have been numerous quantification studies on iron(III) and iron(II), including studies involving gravimetric analysis,[2] titrimetry,[2] electrical analysis,[3] electron spectrophotometry,[3] and inductively coupled plasma emission spectrometry.[2] Methods that employ 1,10-phenanthroline (phen),[4–8] 2,2′-bipyridine (byp),[7–12] and thiocyanate[7,8] are commonly used for spectrophotometry. Moreover, we have already reported the spectrophotometric determinations[10–12] of iron(III) with *o*-hydroxyhydroquinonephthalein (QP). These methods for the determination of iron(III) are highly sensitive, the simultaneous and fractional determination of iron(III) and iron(II) has not yet been studied. This report discusses the fractionation of iron(III) and iron(II) by re-examining the analytical conditions for the determinations of total iron and iron(III) with QP. In addition, we studied the reaction using spectrophotometry and electron spin resonance (ESR).

EXPERIMENTAL

Reagents and Apparatus Stock solutions (1.0×10^{-3} M, 1 M = 1 $mol \cdot l^{-1}$) of ammonium iron(II) sulfate 12-water (Wako Pure Chem. Co. Ltd.) and ammonium iron(III) sulfate hexahydrate (Wako Pure Chem. Co. Ltd.) were prepared in water containing 1 ml of 0.5 M sulfuric acid. Working solutions were prepared by the suitable dilution of these stock solutions as required. A solution of QP, which had been synthesized according to a method described in the literature,[13,14] was prepared in a 1.0×10^{-3} M methanol solution containing one drop of hydrochloric acid. A 2.0% aqueous solution of poly(*N*-vinyl pyrrolidone) (PVP, K-90, Wako Pure Chem. Co. Ltd.) was prepared by dissolving PVP in water. A buffer solution of pH 9.0 was made by mixing appropriate amounts of a 0.2 M bicine (*N*,*N*-bis(2-hydroxyethyl)glycine) solution and a 0.1 M sodium hydroxide solution. Reagent-grade chemicals were used throughout. Just before use, pure water was prepared by purifying deionized water with a Milli-Q

*e-mail: fujitay@gly.oups.ac.jp

Labo system.

A Shimadzu spectrophotometer (Model UV-2400PC) with 1.0-cm matched silica cells was used for the absorbance measurements, and a different Shimadzu spectrophotometer (Model UV-160) was used for the reaction-rate measurements. The pH measurements were made with a Horiba (F-8) pH meter in combination with a calomel glass electrode. A JEOL RE-1X was used to obtain the ESR spectrum.

Standard Procedure for Determination of Total Iron (Iron(III) + Iron(II)) The following components were mixed in a 10-ml volumetric flask: a solution containing 0.2–6.7 μg of iron ions, 2.0 ml of a 2.0% PVP solution, 2.0 ml of the 0.2 M bicine/0.1 M sodium hydroxide buffer solution (pH 9.0), and 1.0 ml of a 1.0×10^{-3} M QP solution. The mixture was diluted to 10 ml with water, transferred to a test tube, mixed well, and kept at room temperature for 5 min. The absorbance of the resultant solution was measured at 570 nm against a reagent blank without iron ions.

Standard Procedure for Determination of Iron (III) Alone The following components were mixed in a 10-ml volumetric flask: a solution containing 0.2–6.7 μg of iron(III), 1.0 ml of a 1.0×10^{-2} M potassium cyanide solution, 2.0 ml of a 2.0% PVP solution, 2.0 ml of the 0.2 M bicine/0.1 M sodium hydroxide buffer solution (pH 9.0), and 1.0 ml of a 1.0×10^{-3} M QP solution. The mixture was diluted to 10 ml with water, transferred to a test tube, mixed well, and kept at 60℃ for 20 min. After the solution had been cooled in water to room temperature, the absorbance of the resultant solution was measured at 570 nm against a reagent blank without iron(III).

RESULTS and DISCUSSION

Effect of Buffer Solution and pH The effects of the pH values of the solutions containing iron(III) and iron(II) on the color reaction were examined using strongly acidic, weakly acidic, neutral, and weakly basic solutions. The coloring intensities and reaction rates of both ions with QP were the highest in the weakly basic solution (Table 1). The effects of the buffer solution on the color reaction were examined by using Good buffers with buffering activities in the weakly basic range. These buffers included bicine/sodium hydroxide and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)/sodium hydroxide, as well as other buffers such as ammonia/ammonium chloride buffer and triethanolamine/hydrochloric acid buffer. Since the 0.2 M bicine/0.1 M sodium hydroxide alone induced similar color intensities in the iron(III) and iron(II), this buffer was the most appropriate for the determination of total iron. Next, the effects of the pH were examined using this buffer. The difference between the absorbance of the iron-QP solution and that of the QP solution was almost constant at pH values between 7.8 and 10.8, but the absorbance of the QP solution increased rapidly at a pH above 10. Figure 1 shows trend of iron(II), it was same as iron(III). Thus, 2.0 ml of the 0.2 M bicine/0.1 M sodium hydroxide solution (pH 9.0) was used for the pH adjustments.

Table 1. Effect of Buffer Solutions and pH

Buffer solution	pH	Absorbance at λ_{max}	
		Fe(II)	Fe(III)
H_2SO_4	1.5	0.051 : 540	0.066 : 540
CH_3COONa/CH_3COOH	3.7	0.400 : 550	0.411 : 550
CH_3COONH_4	6.6	0.227 : 565	0.154 : 560
Bicine/NaOH	8.7	0.651 : 570	0.651 : 570
Tris/HCl	7.8	0.584 : 570	0.411 : 570
BES[a]/NaOH	7.9	0.388 : 570	0.191 : 570
TAPS[b]/NaOH	8.0	0.560 : 570	0.287 : 570
HEPES-NaOH	8.5	0.296 : 570	0.125 : 570
TEAHC[c]/HCl	8.6	0.666 : 570	0.558 : 570
Na_2HPO_4/KH_2PO_4	8.6	0.606 : 570	0.586 : 570
CHES/NaOH	8.8	0.315 : 570	0.185 : 570
NH_3/NH_4Cl	9.2	0.406 : 570	0.347 : 570
$Na_2B_4O_7$	9.3	0.412 : 545	0.450 : 545

Fe ions: 5.0×10^{-6} M; PVP: 0.4%; QP: 1.0×10^{-4} M; Reference: QP solution. [a] *N*,*N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, [b] *N*-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid, [c] Triethanolamine hydrochloride.

Effect of Surfactants The effects of dispersing agents on the iron(III)-QP and iron(II)-QP solutions were examined using cationic surfactants (cetyltrimethylammonium chloride (CTAC), cetylpyridinium chloride (CPC), stearyltrimetylammonium chloride (STAC)), anionic surfactants (sodium dodecylsulfate (SDS), diisooctyl sodium sulfosuccinate (Aerosol OT)), amphoteric surfactants (Swanol AM-101), and non-ionic surfactants (PVP, Brij 35, Triton X-100, Tween 20, methylcellulose) (Table 2). The maximal and constant absorbances were obtained by adding more than 2.0 ml of 2.0% PVP (K-90) to 10 ml of the iron(III)-QP and iron(II)-QP solutions.

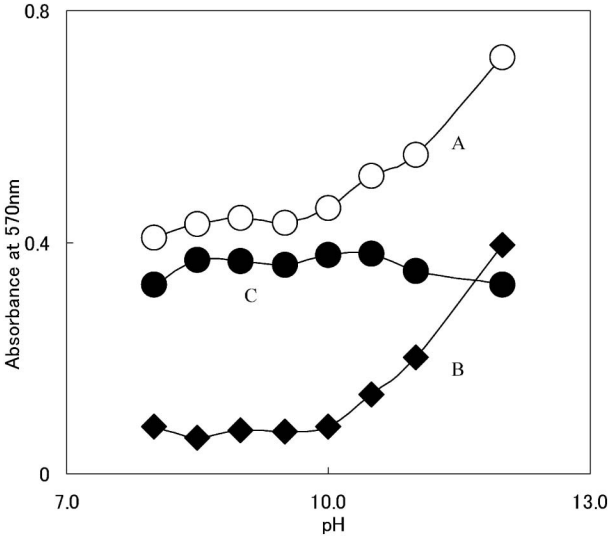

Fig. 1. Effect of pH on Iron Ions

Fe(II) ions: 3.0×10^{-6} M; PVP: 0.4%; QP: 1.0×10^{-4} M; A, Sample solution: B, Blank solution: C, Sample minus blank; Reference: water.

Table 2. Effect of Surfactant

Surfactants	Absorbance at λ_{max}	
	Fe(II)	Fe(III)
None	0.544 : 560	0.545 : 560
PVP (K-90)	0.651 : 570	0.651 : 570
PVP (K-30)	0.645 : 570	0.651 : 570
CPC	0.576 : 590	0.575 : 590
CTAC	0.603 : 590	0.597 : 590
STAC	0.616 : 590	0.610 : 590
Aerosol OT	0.540 : 560	0.537 : 560
SDS	0.544 : 560	0.550 : 560
Swanol AM-104	0.179 : 570	0.196 : 570
Tween 20	0.468 : 560	0.472 : 560
Brij 35	0.478 : 560	0.484 : 560
Triton X-100	0.484 : 560	0.480 : 560
Metylcellulose	0.487 : 560	0.473 : 560

Fe ions: 5.0×10^{-6} M; cationic surfactants: 1.0×10^{-3} M; other surfactants: 0.1–0.4%; pH: 9.0; QP: 1.0×10^{-4} M; Reference: QP solution.

Effect of QP Concentration The optimal QP concentration was studied in the presence of 3.0×10^{-6} M and 5.0×10^{-6} M iron(III) or iron(II). The maximal and constant absorbance was obtained by adding more than 0.6 ml of the 1.0×10^{-3} M QP solution to the final volume of 10 ml. Therefore, the final concentration of the QP solution was determined to be 1.0×10^{-4} M in the final volume.

Stability of Coloration and Effect of Ionic Strength The changes in the absorbance with time were examined in the presence of the bicine buffer and PVP. The absorbance became constant about 10 s after mixing with the reagents, indicating that this color reaction reached equilibrium very rapidly. Examinations using the other buffers under the same conditions indicated that the reaction rate was equally high in these buffers, except for the phosphate buffers. In addition, the surfactants used made no difference at all in the reaction rates. The color reactions of the iron(III) and iron(II) against QP were completely the same. Next, we examined the effects on the absorbance values of the sequence used to add the reagents, and obtained the highest absorbance by keeping the addition of the QP solution until last. Therefore, a 5-min standing time at room temperature was selected for all of the measurements to determine total iron. The possible effects of ionic strength were examined using a 1.0 M potassium chloride solution, and it was found that the absorbance was only slightly reduced even when 3.0 ml of the 1.0 M potassium chloride solution was added to the QP-iron ion solution.

Evaluation of Masking Agents for Fractional Assay of Iron(III) and Iron(II) Ions To establish a fractional quantification method for iron(III) and iron(II) ions, we examined various masking agents. Iron(II) ions were masked with cyanide ions, but the color reaction rates of the QP and iron(III) ions in the presence of cyanide ions were markedly reduced, and the solutions had to stand for more than 1 h at room temperature for these reactions to complete. We examined the effect of heating temperature and time, and the order that the reagents were added, on the iron(III) ion assay, and found that the optimum combination involved additions of iron(III) ions followed by cyanide ions, PVP, buffer, and QP, and heating for 20 min at 60℃. We also investigated the optimum amount of cyanide solution required for the masking agent, and found that complete masking of the iron(II) ions could be obtained by adding more than 0.5 ml of the 1.0×10^{-2} M potassium cyanide solution to the final volume of 10 ml, as shown in Fig. 2. On the other hand, both the iron(III) and iron(II) ions were completely masked by the addition of an aminopolycarboxylic acid such as EDTA or EDTA-OH. Both ions were masked to a certain degree by the addition of phen or byp and could not be masked by the addition of fluoride ions or acetylacetone. When nitrite ions were added, the absorbance of the iron(III) ions was rapidly reduced, but the color intensities of the iron(III) and iron(II) ions became similar

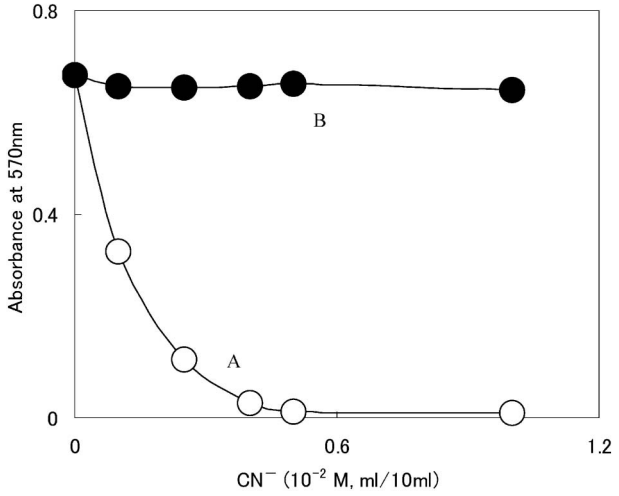

Fig. 2. Effect of Cyanide Ion Concentrations

Fe ions: 5.0×10^{-6} M (A: Fe(II); B: Fe(III)); PVP: 0.4%; pH: 9.0; QP: 1.0×10^{-4} M; Reference: QP solution.

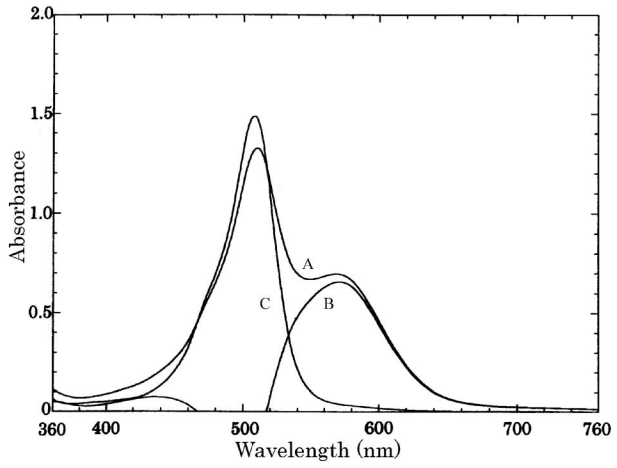

Fig. 3. Absorption Spectra

Fe(II), Fe(III): 5.0×10^{-6} M; PVP: 0.4%; pH: 9.0; KCN: 10×10^{-2} M; QP: 1.0×10^{-4} M. Curve A, Fe(II)-QP and Fe(III)-QP solutions in the absence of KCN; Curve B, Fe(II)-QP solution in the presence of KCN and QP solution; Curve C, Curve A minus Curve B; Reference: Water.

Table 3. Effect of Masking Reagent on Iron(II) or Iron(III)

Masking regent	(M)	Absorbance at 570 nm	
		Fe(II)	Fe(III)
KCN	1.0×10^{-2}	0	0.390
	1.0×10^{-3}	0.390	0.390
EDTA	1.0×10^{-2}	0	0
	1.0×10^{-3}	0	0
EDTA-OH	1.0×10^{-2}	0	0
	1.0×10^{-3}	0	0
Acetylacetone	1.0×10^{-2}	0.356	0.307
	1.0×10^{-3}	0.375	0.386
NaF	1.0×10^{-2}	0.390	0.374
	1.0×10^{-3}	0.390	0.390
$NaNO_2$	1.0×10^{-2}	0.377	0.341
	1.0×10^{-3}	0.390	0.351
phen	1.0×10^{-2}	0.121	0.117
	1.0×10^{-3}	0.180	0.170
byp	1.0×10^{-2}	0.206	0.196
	1.0×10^{-3}	0.322	0.343

Fe ions: 3.0×10^{-6} M; PVP: 0.4%; pH: 9.0: 2.0 ml; QP: 1.0×10^{-4} M; Reference: QP solution.

through heating. The results are shown in Table 3.

Absorption Spectra According to the standard procedures, the absorption spectra of the iron(III)-QP, iron(II)-QP, and QP solutions were measured with and without potassium cyanide solution. As shown in Fig. 3, the absorption spectra of the iron(III)-QP and iron(II)-QP solutions were identical without potassium cyanide, even when hydrogen peroxide and an ascorbic acid solution were added to the iron(III)-QP and iron(II)-QP solutions, respectively. On the other hand, the iron(II) ions were completely masked with the potassium cyanide solution (1.0×10^{-2} M). The absorption spectra of the iron(II)-QP and QP solutions were very similar in the presence of the potassium cyanide solution.

Calibration Curve, Sensitivity, and Accuracy On the calibration curve produced using the standard procedure, a good linear relationship was observed for 0.02–0.67 $\mu g\cdot ml^{-1}$ of iron ions. The effective molar absorptivity (ε) of the iron(III)-QP and iron(II)-QP solutions and of the iron(III)-QP solution in the presence of the cyanide ion solution was $1.30\times10^{5}\cdot l\ mol^{-1}\cdot cm^{-1}$. The sensitivity of this method was more than 10-fold higher than that of the phen method ($\varepsilon=1.1\times10^{4}\cdot l\ mol^{-1}\cdot cm^{-1}$), about 10-fold higher than that of the thiocyanate method ($\varepsilon=1.4\times10^{4}\cdot l\ mol^{-1}\cdot cm^{-1}$), and more than 15-fold higher than that of the byp method ($\varepsilon=7.9\times10^{3}\cdot l\ mol^{-1}\cdot cm^{-1}$). The ε of the proposed method was slightly higher than the other methods, which used xanthene dyes,[10,11] and the reaction rate was extremely rapid. On the other hand, the published methods cannot be used to determine total iron or iron(III) alone. The fact that the sensitivities of the iron(II)-QP and iron(III)-QP complexes were identical confirmed that the recommended procedures described in the literature reflect the total concentration of iron

ions. The relative standard deviations (RSD) of all of the procedures were below 0.77% ($n=8$), indicating that the reproducibility of the proposed method was good.

Effects of Coexisting Ions The effects of coexisting metal ions on the proposed method were examined by using 0.17 $\mu g \cdot ml^{-1}$ iron(III) and iron(II). At molar quantities greater than 100-fold those of the iron(III) and iron(II) ions, there were no effects from sodium(I), potassium(I), calcium(II), magnesium(II), cadmium(II), palladium(II), selenium(IV), platinum(IV), germanium(IV), or chromium(VI). At molar quantities equal to those of iron(III) and iron(II), there were no effects from copper(II), nickel(II), manganese(II), magnesium(II), tin(II), lanthanum(III), zirconium(IV), hafnium(IV), vanadium(V), or molybdenum(VI). Very large positive or negative errors were observed with zinc(II), lead(II), indium(III), aluminum(III), bismuth(III), thorium(IV), titanium(IV), niobium(V), or osmium(VIII). Among these interfering metal ions, the effects of zirconium(IV) and molybdenum(VI) could be eliminated using nitrilotriacetic acid (NTA) (3.0×10^{-3} M), those of zinc(II), zirconium(IV), hafnium(IV), and molybdenum(VI) could be eliminated using iminodiacetic acid (IDA) (3.0×10^{-3} M), and those of lanthanum(III) could be eliminated using sodium fluoride (3.0×10^{-3} M). The effects of anions were also examined. These had no influence at all in the presence of fluoride, bromide, iodide, phosphate, sulfate, nitrate, and thiocyanate ions, even in large amounts. The results are summarized in Table 4.

Table 4. Effect of Foreign Ions and Selection of Masking Agents

Substance (+Masking Regent)	Molar ratio (Substance /Fe ions)	Absorbance at 570 nm	Recovery (%)
None	100	0.390	100.0
Na(I)	100	0.390	100.0
K(I)	100	0.390	100.0
Ca(II)	100	0.390	100.0
Mg(II)	100	0.390	100.0
Pd(II)	100	0.390	100.0
Cd(II)	100	0.390	100.0
Co(II)	10	0.423	108.5
Ni(II)	100	0.461	118.3
Mn(II)	100	0.524	134.4
Cu(II)	5	0.445	114.0
Zn(II)	100	0.523	134.0
Zn(II)+IDA*	100	0.390	100.0
Pb(II)	10	0.527	135.2
La(III)	10	0.408	104.6
$La(III)^{+}+F^{-}$*	100	0.390	100.0
In(III)	1	0.538	138.0
Al(III)	1	0.447	114.7
Bi(III)	5	0.512	131.3
Se(IV)	100	0.390	100.0
Pt(IV)	100	0.390	100.0
Ge(IV)	100	0.390	100.0
Zr(IV)	100	0.523	134.0
Zr(IV)+NTA*	100	0.390	100.0
Hf(IV)	100	0.321	82.2
Hf(IV)+IDA*	100	0.390	100.0
Ti(IV)	1	0.613	157.2
Th(IV)	5	0.518	132.7
V(V)	100	0.484	124.0
Nb(V)	5	0.531	136.9
Cr(VI)	100	0.390	100.0
Mo(VI)	100	0.450	115.4
Mo(VI)+NTA*	100	0.390	100.0
Os(VIII)	5	0.560	143.5
NaF	100	0.390	100.0
NaBr	100	0.390	100.0
NaI	100	0.390	100.0
Na_2HPO_4	100	0.390	100.0
Na_2SO_4	100	0.390	100.0
$NaNO_3$	100	0.390	100.0
KSCN	100	0.390	100.0
NTA	100	0.390	100.0
IDA	100	0.390	100.0

Fe ions: 3.0×10^{-6} M; PVP: 0.4%; pH: 9.0: 2.0 ml; QP: 1.0×10^{-4} M; Reference: QP solution. * Masking reagent: 3.0×10^{-3} M.

Evaluation of the Proposed Method in Pharmaceutical Preparations This method was applied to the assays of iron(III) and iron(II) in pharmaceutical preparations, and the analytical values and recoveries were determined. The contents of tablets or a capsule were accurately weighed and ground in a mortar to a fine powder. The requisite volume of powder was weighed, transferred to a 100-ml volumetric flask, diluted to the mark with 0.1 M sulfuric acid, and filtered. An appropriate amount of the sample solution was taken and determined according to the standard procedure. The recovery of each sample was calculated by comparing the concentration obtained from the spiked mixtures with those of iron ions. As shown in Table 5, the analytical values and recoveries determined for all of the samples by this method were similar to or better than those obtained by the phen method,[15] indicating that this method is valuable for practical use. It was confirmed that iron(III) and iron

Table 5. Total Iron and Iron(III) in Pharmaceutical Preparations

Sample	Amount of total iron (mg)[a]			Amount of iron (III) (mg)[a]	
	Nominal	Found (Recovery (%))[b]		Found (Recovery (%))[b]	
		This method	Phen method[c]	This method	Phen method[c]
A	50	50.1 (102.3)	49.7 (101.5)	9.6 (102.1)	8.6
B	100	99.3 (101.2)	99.0 (100.3)	14.5 (101.6)	9.7
C	50	49.0 (99.0)	50.8 (101.2)	10.6 (103.1)	8.1
D	105	105.4 (96.2)	104.4 (100.3)	2.7 (101.1)	2.7
E	100	102.6 (100.5)	103.2 (100.6)	5.4 (101.5)	7.0
F	40	41.9 (99.3)	40.4 (101.4)	41.6 (102.1)	37.8
G	2	1.7 (102.8)	1.7 (102.2)	1.6 (99.6)	1.5

Sample A, Sodium Ferrous Citrate (tablet); Sample B, Sodium Ferrous Citrate (granule); Sample C, Sodium Ferrous Citrate (tablet); Sample D, Ferrous Sulfate (tablet); Sample E, Ferrous Fumarate (capsule); Sample F, Chondroitin Sulfate-Iron Colloid (ampule); Sample G, Iron(III) Pyrophosphate (tablet, food). [a] mg for 1 tablet, 1 package, 1 capsule, and 1 ampule. [b] The average of 5 determinations. [c] Total iron minus iron(II) by phen method.

Table 6. The Composition of Iron-QP Complexes[a] in Various Buffer Solutions with and without PVP

Color reaction Condition Buffer	pH	60℃ for 15 min with PVP		Room temp. for 20 min with PVP		Room temp. for 20 min without PVP	
		Fe(II) : QP	Fe(III) : QP	Fe(II) : QP	Fe(III) : QP	Fe(II) : QP	Fe(III) : QP
Bicine/NaOH	9.0	1 : 3	1 : 3	1 : 3	1 : 3	1 : 1	1 : 1
NH_3/NH_4Cl	9.3	1 : 2	1 : 1	1 : 2	1 : 2	3 : 1	3 : 1
KH_2PO_4/Na_2HPO_4	8.7	1 : 2	1 : 2	1 : 2[b]	1 : 2[b]	1 : 2	3 : 1
Tris/HCl	8.7	1 : 2	1 : 1	1 : 3	1 : 1	1 : 2	1 : 1
HEPES/NaOH	9.0	1 : 2	1 : 3	1 : 2	1 : 2	1 : 2	3 : 1
CHES/NaOH	9.1	1 : 2	1 : 3	1 : 3	1 : 2	1 : 2	3 : 1

[a] Determined by continuous variation and mole ratio methods, [b] 40℃ 10 min.

(II) coexisted in each sample. However, the pretreatment in this method requires further examination.

Composition of Coloring Complex Under the obtained condition of determination, the influence of PVP on the coloring complex was evaluated. The composition ratios of iron(III) or iron(II) to QP were examined with different buffers in the presence or absence of PVP by the continuous variation and molar ratio method, respectively. The molar ratios of both iron(III) to QP and iron(II) to QP were 1:3 with bicine/sodium hydroxide buffer in the presence of PVP and 1:1 in the absence of PVP. The composition was also examined with other buffers, as shown in Table 6. Interestingly, the composition ratio of the iron(III) or iron(II) to QP differed considerably with the buffer used.

Evaluation of Reaction Pattern of Iron(III) and QP by ESR Because QP is a catechol-type compound with adjoining hydroxyl residues in the structure, it is suggested that an oxidation-reduction reaction occurs between iron(III) and catechol in the QP structure. To evaluate the reaction pattern, the reactivity between iron(III) and QP was examined using the electron spin resonance (ESR). The ESR signal of the iron(III)-QP complex measured at 77 K was 150 mT, and the g value was a single signal close to 4.4, indicating that iron(III) did not reduce to iron(II) by QP but existed as the iron(III)-QP complex (Fig. 4). These results indicated that the iron(II)-QP and iron(III)-QP complexes coexisted in the presence of both iron(III) and iron(II) and that their ε values were equal.

CONCLUSION

We improved a simple and highly sensitive spectrophotometric method for the determination of total iron by using QP in the presence of bicine/sodium hydroxide buffer and PVP. With this method, good analytical values and recoveries were obtained from assays of total iron in pharmaceutical preparations,

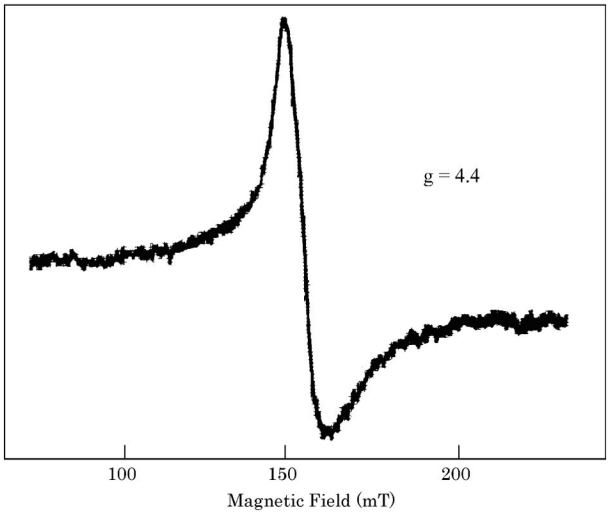

Fig. 4. ESR Spectrum
QP:Fe=20:1 (Fe 100 μM); Temperature: 77 K; Power: 5 mW; Field: 150±100 mT; Sweep time: 8 min/200 mT; Mod.: 0.63 mT; Receiver gain: 5 ×100; Time constant: 0.1 s.

indicating that the method is suitable for practical use. Since iron(III) alone could be quantified by adding a potassium cyanide solution, the possibility that this method can also be used for the fractional quantification of iron(III) and iron(II) in actual materials was shown. However, the use of masking agents in this method requires further examination.

The molar ratios of the iron-QP complexes generated by this reaction were iron(III):QP=1:3 and iron(II):QP=1:3 in the presence of PVP, and iron(III):QP=1:1 and iron(II):QP=1:1 in the absence of PVP, suggesting the high involvement of the bicine buffer and PVP used for the color reaction. The generation of the iron(III)-QP complex was confirmed by ESR. Interestingly, the coexistence of the iron(III)-QP complex and iron(II)-QP complex with equal levels of coloration was also confirmed.

REFERENCES

1) Cotton, F. A., G. Wilkinson, "Advanced Inorganic Chemistry: A Comprehensive Text," 4th ed. John Wiley & Sons, New York, 1980, p. 745.
2) The Japan Society for Analytical Chemistry, "Bunsekikagaku Binran," Maruzen, Tokyo, 1967, p. 339.
3) Plambeck J. A., "Electroanalytical Chemistry," John Wiley & Sons, New York, 1982, p. 297.
4) Blau F., *Monatsh.*, **19**, 647 (1898).
5) Saywell L. G., Cunningham, B. B., *Ind. Eng. Chem., Anal. Ed.*, **9**, 67 (1937).
6) Wenger P., Duckert R., *Helv. Chim. Acta*, **27**, 757 (1944).
7) Sandell E. B., "Colorimetric Determination of Trace of Metal," 3rd ed., Interscience Publishers Inc., New York, 1959, p. 524.
8) Rao T. P., Reddy M. L. P., Pillai A. R., *Talanta*, **46**, 765–813 (1998).
9) Feigl F., Hamburg H., *Z. Anal. Chem.* **86**, 7 (1931).
10) Fujita Y., Mori I., Fujita K., Nakahashi Y., *Chem. Pharm. Bull.*, **1**, 254 (1988).
11) Mori I., Fujita Y., Toyoda M., Kashiwagi M., *Fresenius J. Anal. Chem.*, **340**, 57–59 (1991).
12) Fujita Y., Mori I., Enoki T., *Bunseki Kagaku*, **24**, 253 (1975).
13) Emara H. M., Allen H. F. "Organic Syntheses. Collective Vol. 1," John Wiley & Sons, New York, 1967, p. 317.
14) Liebermann C., Lindenbaum S., *Chem. Ber.*, **37**, 1171 (1901).
15) "Mukiouyouhisyokubunseki 2," Kyoritsu Syuppan, Tokyo, 1974, p. 334.