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Approach to Analytical Chemistry of Saponins$^{1)}$: Application of Saponin as a Surfactant Having Complex-Forming Ability to Spectrophotometry of Iron Ion

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An attempt was made to apply saponin to analytical spectrophotometry; color reactions between *o*-hydroxyhydroquinonephthalein (Qnph) and several metal ions in the presence of surfactants including saponin were studied in weakly basic media. A simple, sensitive and selective spectrophotometric determination of iron ion (iron^{III}+iron^{II}) was developed with Qnph and saponin. The apparent molar absorptivity for iron ion was 1.18×10^5 l mol^{-1} cm^{-1} at 565 nm. It was suggested that saponin is a surfactant having complex-forming ability with metal ions, and iron ion combined with hydrophilic sugar groups in the saponin. The proposed method was applied to the assay of iron ion in rain water, tap water, human urine and calf serum, and the analytical results were in good agreement with those obtained by atomic absorption spectrometry, inductively coupled plasma atomic emission spectroscopy and spectrophotometry with bathophenanthroline.

Keywords——saponin; complex-forming ability; surfactant; iron ion; *o*-hydroxyhydroquinonephthalein; spectrophotometry

In recent years the use of surfactants, particularly cationic surfactants such as cetyltrimethylammonium chloride (CTAC) and cetylpyridinium chloride (CPC), has been proposed for the development of new spectrophotometric methods for assay of various metal ions.$^{2)}$ The introduction of these surfactants in analytical chemistry has led to some advantages such as: (i) a lowering of the pH area at which the complex is formed; (ii) a bathochromic shift in the absorption band; (iii) an increase in molar absorptivity; (iv) stabilization of the complex formed; (v) solubilization of the complex formed; (vi) an increase of reaction rate; (vii) an increase in selectivity. Thus, the use of new surfactants is expected to aid the development of superior methods for the determination of metal ions.

Saponins$^{3)}$ are widely distributed in plants and have recently attracted considerable interest as biologically active substances. Although investigations of the structural features and biological activities of saponins have been reported, little work$^{4)}$ has been done on fundamental or application studies of saponins, or on analytical applications of saponins. Generally, saponins have a hydrophobic surface (aglycones: triterpene or steroid) and a hydrophilic surface (sugar groups), and therefore they have an ability to form micelles. Thus, as the hydrophilic sugar groups having O donor atoms (neutral O atoms) are exposed to water in aqueous solution, it is speculated that saponins can form complexes with various metal ions.

We examined an analytical application of saponins as surfactants having complex-forming ability with metal ions, and favorable results were obtained in the color reaction between *o*-hydroxyhydroquinonephthalein (Qnph) as an organic reagent and iron ion (iron^{III}+iron^{II}) as a metal ion in the presence of saponin. Fundamental conditions, therefore, for the spectrophotometric determination of iron ion were investigated by using Qnph and saponin, and the proposed method was then applied to the assay of iron ion in a variety of samples (rain water, tap water, human urine and calf serum).

Experimental

Reagents and Materials——Standard solutions (1.0×10^{-2} M, M = mol l^{-1}) of iron(III) and iron(II) were prepared by dissolving proper quantities of ammonium iron(III) sulfate 12 hydrate and ammonium iron(II) sulfate 6 hydrate, respectively, in 5 ml (ml = cm^3) of 1.0 M sulfuric acid and diluting the mixture to 100 ml with water. The iron(II) solution was prepared as required. The working solutions were made by suitable dilution of these standard solutions as required. A Qnph solution was prepared by the published procedure[5] in methanol containing one drop of hydrochloric acid to give a 1.0×10^{-3} M solution. A 2.0% saponin solution was prepared by dissolving saponin (Merck, white pure, Art. 7695) without purification with hot water and stored in a cool place. A buffer of pH 9.3 was prepared by mixing 0.2 M ammonia and 0.2 M ammonium chloride solutions. All chemicals used were of analytical reagent grade unless otherwise stated. Deionized water was used in the preparation of all solutions.

Apparatus——Absorption spectra and absorbance values were measured with a Shimadzu model 240 automatic recording digital spectrophotometer using 10-mm quartz cells. A Hitachi-Horiba model F7 AD pH meter equipped with a combined glass electrode was used for the pH measurements. Atomic absorption measurements were made on a Shimadzu model AA-670 atomic absorption spectrometer with a air-acetylene flame. A Shimadzu model ICPS-1000V inductively coupled plasma emission spectrometer was used.

Standard Procedure for the Spectrophotometry of Iron Ion——A solution containing up to 6 μg of iron ion was transferred to a 10-ml volumetric flask; to this solution, 1.0 ml of a 2.0% saponin solution, 3.0 ml of the buffer solution (pH 9.3) and 1.5 ml of a 1.0×10^{-3} M Qnph solution were added. The mixture was diluted to 10 ml with water and kept at room temperature for 10 min, then the absorbance of the Qnph–iron solution was measured at 565 nm against a Qnph solution (reagent blank).

Results and Discussion

Color Reaction

In order to examine the effect of saponin on the color reaction between an organic reagent and a metal ion, Qnph (a xanthene dye) was selected as the reagent in this experiment. Iron(III), aluminum(III), copper(II), manganese(II) and cobalt(II) were chosen as metal ions since they form stable complexes with Qnph[6] in basic media.

The color reactions between Qnph and these metal ions in the presence or absence of various surfactants were examined at room temperature. Surfactants used were CTAC (cationic), sodium dodecyl sulfate (SDS, anionic), polyoxy (ethylene) sorbitan monolaurate (Tween 20, nonionic) and saponin. In the presence of saponin, the color reaction between Qnph and iron(III) was remarkably promoted, whereas that between Qnph and aluminum(III), copper(II), manganese(II) or cobalt(II) was considerably depressed, as shown

TABLE I. Effect of Some Surfactants Including Saponin on the Color Reaction between Qnph and Several Metal Ions[a]

Surfactant	Absorbance difference[b] at λ_{max}/nm				
	Fe(III)	Al(III)	Cu(II)	Co(II)	Mn(II)
None	0.070 (535)	0.592 (535)	0.440 (535)	0.365 (545)	0.392 (535)
Tween 20	0.082 (560)	0.645 (535)	0.452 (535)	0.430 (545)	0.290 (535)
CTAC	0.112 (580)	0.680 (545)	0.688 (550)	0.732 (580)	0.755 (555)
SDS	0.100 (560)	0.588 (535)	0.482 (535)	0.300 (555)	0.320 (535)
Saponin	0.590 (565)	0.342 (545)	0.163 (545)	0.165 (545)	0.166 (545)

a) Metal ion, 5.0×10^{-6} M; Qnph, 1.5×10^{-4} M; surfactant, 0.2%[c]; pH, 9.3; reference, water. *b*) Difference of absorbance between Qnph–Fe(III) and Qnph solutions. *c*) This concentration is above the cmc.[2]

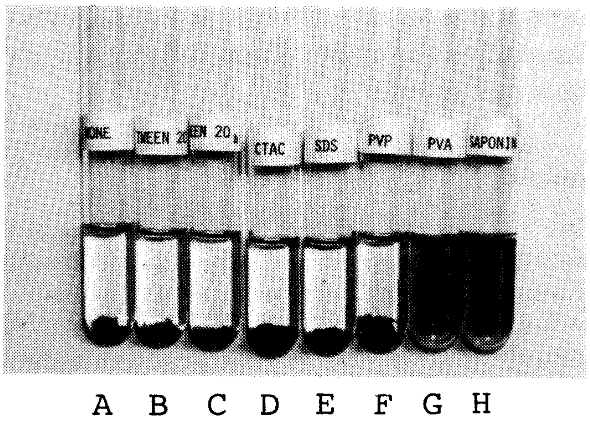

Fig. 1. Qualitative Tests of Fe(III) in the Presence of Surfactants in Test Tubes

Fe(III), 560 μg; pH, 9.0.
A, surfactant none; B, Tween 20 (0.2%)[a]; C, Tween 20 (1.0%); D, CTAC (0.2%); E, SDS (0.2%); F, PVP (0.2%); G, PVA (0.2%); H, saponin (0.2%).
a) This surfactant concentration is above the critical micelle concentration (cmc).[2]

TABLE II. Effect of Surfactants

Surfactant	Absorbance difference[a] at λ_{max}/nm	Surfactant	Absorbance difference[a] at λ_{max}/nm
None	0.070 (535)	LS	0.058 (565)
Saponin	0.590 (565)	PEG 4000	0.062 (565)
Dextran 70	0.472 (555)	PVA	0.248 (565)
Acacia	0.362 (560)	PVP	0.052 (570)
CMC	0.164 (555)	Gelatin	0.102 (570)
Sodium arginate	0.192 (555)	Tween 20	0.082 (560)
CTAC	0.112 (580)	Triton X 100	0.123 (560)
CPC	0.080 (580)	Brij 35	0.047 (565)
SDS	0.100 (560)		

a) Difference of absorbance between Qnph–Fe(III) and Qnph solutions. Fe(III), 5.0×10^{-6} M; Qnph, 1.5×10^{-4} M; surfactant, 0.2%; pH, 9.3; reference, water. CMC, sodium carboxymethyl cellulose; CPC, cetylpyridinium chloride; LS, sodium *N*-lauroylsarcosine; PEG, polyethyleneglycol; PVA, polyvinyl alcohol; PVP, polyvinyl pyrrolidone; Triton X 100, polyethylene glycol mono-*p*-isooctylphenyl ether; Brij 35, polyoxyethylene monolauryl ether.

in Table I. On the other hand, qualitative tests for iron(III) (large amounts of iron (III)) in the presence or absence of various surfactants without Qnph were examined in weakly basic media in test tubes. As shown in Fig. 1, iron(III) was precipitated as a hydroxide even in the presence of a surfactant such as Tween 20, CTAC, SDS, or polyvinyl pyrrolidone (PVP), but no formation of iron(III) hydroxide was observed in the presence of saponin or polyvinyl alcohol (PVA). Similar phenomena were observed when copper(II) was used instead of iron(III). However, aluminum(III), manganese(II) or cobalt(II) did not produce a precipitate only in the presence of saponin. Moreover, Shirai *et al.*[7] reported that iron(III) or copper(II) formed chelate compounds with PVA in aqueous solution. The absorbance of the Qnph–iron solution in the presence of various surfactants was measured and the results are shown in Table II.

It was recognized that saponin has complex-forming ability with metal ions and should be effective for the determination of iron ion with an organic reagent such as Qnph. Hence, the fundamental conditions for the spectrophotometric determination of iron ion by using Qnph and saponin were examined in subsequent experiments.

Study for the Spectrophotometry of Iron Ion

Figure 2 shows the absorption spectra of the Qnph–iron(III) and Qnph solutions in the presence of saponin. The maximum difference of absorbance between the Qnph–iron(III) and Qnph solutions was observed at about 565 nm when Qnph was in large excess over iron(III).

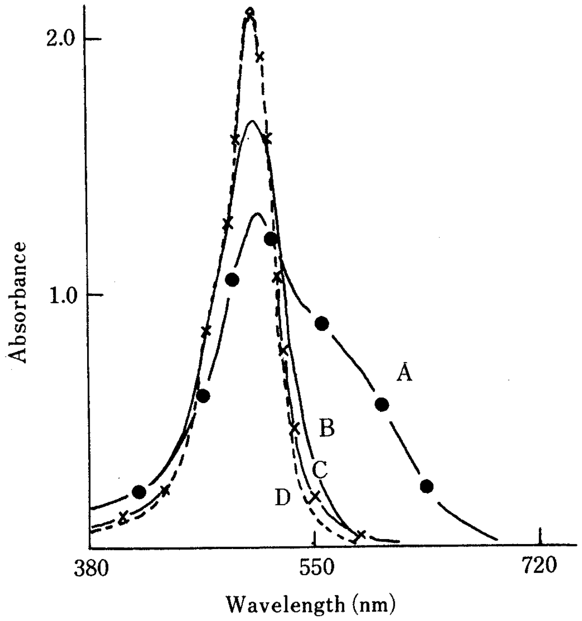

Fig. 2. Absorption Spectra of Qnph–Fe(III) and Qnph Solutions in the Presence or Absence of Saponin

Fe(III), 8.0×10^{-6} M; saponin, 0.2%; Qnph, 4.0×10^{-5} M; pH, 9.3; reference, water.

Curve A (—●—), Qnph–Fe(III)–saponin solution; curve B (——), Qnph–saponin solution; curve C (—×—), Qnph–Fe(III) solution; curve D (------), Qnph solution.

The absorption spectra of the Qnph–iron(II) solution was quite similar to that of the Qnph–iron(III) solution. Thus the absorbance measurement for the determination of iron ion was carried out at 565 nm.

The effect of pH was examined by measuring the absorbance at 565 nm, and a maximum and constant absorbance was obtained between pH 8.6 and 10.4. For the determination of iron ion, pH 9.3 was adopted (addition of 3.0 ml of 0.2 M ammonia–0.2 M ammonium chloride buffer solution).

Several preparations of saponin were studied. Saponins used were as follows: derived from *Gosophila penicula* (Merck), *Cameria sinensis* (Wako), *Quillaja saponaria* (Maruzen), *Panax ginseng, Gynostemma pentaphyllum*, and glycyrrhizin (Maruzen). Though some difference of sensitivities was observed, the color reactions between Qnph and iron ion were clear in all saponin micellar media. A constant absorbance of Qnph–iron solution was observed on addition of more than 0.25 ml of 2.0% saponin (Merck) solution in a final volume of 10 ml.

The effect of Qnph amount on the color development was examined by measuring the absorbance at 565 nm, other variables being kept constant. It was found that more than 0.4 ml of 1.0×10^{-3} M Qnph solution sufficed. Hence, 1.5 ml of a 1.0×10^{-3} M Qnph solution was adopted in the final volume of 10 ml. The formation of the Qnph–iron complex was completed instantaneously at room temperature (10—30 °C), and the absorbance was constant for at least 2 h. Therefore, a 10 min standing time at room temperature was selected for all further work.

Calibration Curve, Sensitivity and Precision

A calibration curve for iron ion was constructed by the standard procedure. Beer's law held over the range up to 6 μg of iron ion in the final volume of 10 ml. The apparent molar absorptivity and the Sandell sensitivity were 1.18×10^5 l mol^{-1} cm^{-1} and 0.00047 μg cm^{-2} at 565 nm, respectively. This procedure is much more sensitive than the methods using bathophenanthroline (bathophen, molar absorptivity $\varepsilon = 2.24 \times 10^4$),[8a)] xylenol orange ($\varepsilon = 2.7 \times 10^4$)[8b)] 2,4,6-tris(2′-pyridyl)-*s*-triazine ($\varepsilon = 2.41 \times 10^4$),[8c)] phenyl-2-pyridylketoxime ($\varepsilon = 1.56 \times 10^4$),[8d)] and thiocyanate ($\varepsilon = 1.88 \times 10^4$),[8d)] but slightly less sensitive than the methods using eriochrome cyanine R–CTAC ($\varepsilon = 1.28 \times 10^5$),[9)] chromazurol S–CTAC ($\varepsilon = 1.35 \times 10^5$)[9)] and Qnph in phosphate buffer media ($\varepsilon = 1.7 \times 10^5$).[6a)]

The relative standard deviation was 0.33% for ten runs with 2.8 μg of iron(III).

Influence of Diverse Ions

The influence of diverse ions on the determination of iron(III) was investigated under the optimal conditions. Commonly encountered ions were added individually to a solution containing 2.7 μg of iron(III). The following ions did not interfere: up to 100-fold molar ratio of molybdenum(VI), chromium(VI), vanadium(V), tungsten(VI), *etc.*; 2- to 10-fold molar ratio of thorium(IV), germanium(IV), antimony(III), lanthanum(III), and mercury(II); equimolar zinc(II), cadmium(II), and lead(II). The interference from the large amounts of bivalent metal ions except lead(II)—in about 100-fold molar excess over iron(III)—could be overcome by addition of iminodiacetic acid (IDA) or nitrilotriacetic acid (NTA) as a masking agent; IDA was superior to NTA. On the other hand, most anions except cyanide ion, such as fluoride, citrate, thiocyanate, phosphate, perchlorate, tartrate, sulfide, *etc.* did not interfere in 500- to 1000-fold excess over iron(III). Since iron(II) was influenced by cyanide ion, separative determination of iron(III) and iron(II) might be achieved by further investigation. The results obtained are summarized in Table III.

TABLE III. Effect of Foreign Ions in the Determination of Iron(III)

Foreign ion	Added (μg)	Mole ratio[a]	Absorbance at 565 nm		
			Method A	Method B	Method C
—	—	—	0.590	0.590	0.536
Cu(II), nitrate	3.2	1	0.658	0.590	0.536
	3.2×10^2	100	—	0.590	0.643
Co(II), nitrate	2.9	1	0.646	0.590	
	2.9×10^2	100	—	0.590	
Ni(II), nitrate	2.9	1	0.644	0.590	
	2.9×10^2	100	—	0.590	
Zn(II), nitrate	1.6×10	5	0.642	0.590	
	3.7×10^2	100	—	0.590	
Cd(II), nitrate	2.8×10	5	0.637	0.590	
Hg(II), nitrate	1.0×10^2	10	0.521	0.590	
Mn(II), chloride	5.5	2	0.703	0.590	
Pb(II), chloride	2.1×10	2	0.627		0.536
Ca(II), chloride	2.0×10^2	100	0.590		
Bi (III), nitrate	2.1×10	2	0.642		0.545
La(III), chloride	7.9×10	10	0.600		
Al(III), chloride	1.3	1	0.633		
Sb(III), sulfate	6.1×10	10	0.590		
Sn(IV), sulfate	1.2×10	2	0.549		
Zr(IV), nitrate	2.3×10	5	0.539	0.590	
Ge(IV), chloride	3.6×10	10	0.590		
Th(IV), nitrate	1.2×10^2	10	0.528	0.598	
Ti(IV), sulfate	2.4	1	0.633		
V(V), metavanadate	2.5×10^2	100	0.590		
As(V), arsenate	3.7×10^2	100	0.590		
U(VI), nitrate	2.4×10	2	0.633		
Mo(VI), molybdate	4.8×10^2	100	0.590		
W(VI), tungstate	9.2×10^2	100	0.590		
Cr(VI), dichromate	2.6×10^2	100	0.590		

Iron, 2.7 μg/10 ml; Qnph, 1.5×10^{-4} M; saponin, 0.2%; pH, 9.3; reference, reagent blank. *a*) Foreign ion/iron. Method A; masking agent, none. Method B; IDA (5.0×10^{-3} M) as masking agent. Method C; NTA (1.0×10^{-3} M) as masking agent.

Complex Stoichiometry

The continuous variation method was employed to establish the stoichiometry of the Qnph–iron(III) complex in the presence of saponin. The result indicated that a 2:3 complex between iron(III) and Qnph was formed. This result was confirmed by the molar ratio method and the McConnel–Davidson method.[10)] On the other hand, it was found that the molar ratios of iron(III) to Qnph were approximately 2:1 in the presence of CTAC, Tween 20, or SDS. The results obtained by the continuous variation method in the presence of saponin or CTAC are shown in Fig. 3.

Next, a study to disclose the character and the nature of the saponin was performed. In the first place, as cyanide ion forms a very stable complex with iron ion, the effect of the sequence of addition of cyanide ion on the absorbance of the Qnph–iron solution was examined. As is shown in Table IV, the influence of cyanide ion was reduced when cyanide ion was added to the mixture containing iron ion and saponin. However, the absorbance of the Qnph–iron solution in the presence of other surfactants such as Tween 20 and CTAC was scarcely affected even when the order of addition of cyanide ion was modified. In the second place, the color reaction between Qnph and iron ion was examined when the carbohydrate ingredients of saponin were used instead of saponin. The carbohydrates used were glucuronic

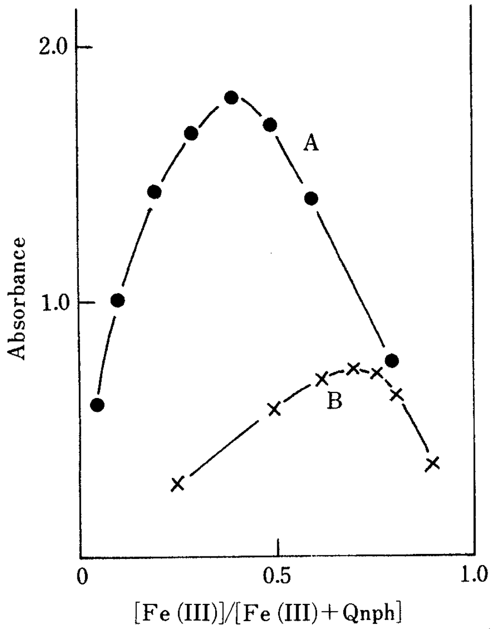

Fig. 3. Composition of [Fe(III):Qnph] in the Presence of Saponin or CTAC, Obtained by the Continuous Variation Method

[Fe(III)+Qnph] = 1.0×10^{-4} M; saponin or CTAC, 0.2%; pH, 9.3; reference, Qnph solution.

Curve A (—●—), in the presence of saponin (at 565 nm); curve B (—×—), in the presence of CTAC (at 580 nm).

TABLE IV. Effect of Sequence of Addition of Cyanide Ion (CN^-) on Absorbance of Qnph–Iron Solution

Sequence of addition	Absorbance of Qnph–iron solution at 565 nm	
	Fe(III)	Fe(II)
Fe→Sap→Buff→Qnph (CN^- none)	0.590	0.590
Fe→CN^-→Sap→Buff→Qnph	0.043	0.015
Fe→Sap→CN^-→Buff→Qnph	0.512	0.060
Fe→Sap→Buff→CN^-→Qnph	0.562	0.554
Fe→Sap→Buff→Qnph→CN^-	0.590	0.578

Iron ion, 5.0×10^{-6} M; Qnph, 1.5×10^{-4} M; saponin, 0.2%; CN^-, 5.0×10^{-3} M; pH, 9.3; reference, reagent blank. Sap, saponin; Buff, buffer solution.

acid, arabinose, galactose, rhamnose, xylose and glucose. The results indicated that the color development in the presence of each carbohydrate was almost identical to that in the presence of saponin. The reason why the absorption bands of the Qnph–iron and Qnph solutions are somewhat red shifted is probably the interaction between Qnph and saponin micelles. However, the color development in the presence of carbohydrate was slow compared with that in the presence of saponin. Iron(III) in the presence of carbohydrate produced a precipitate in the qualitative tests mentioned above. Moreover, in the presence of glycyrrhetinic acid, which is the aglycone of glycyrrhizin, the colored species was scarcely produced. The absorbance of the Qnph–iron solution and the composition of [iron(III): Qnph] formed in the presence of these carbohydrates are given in Table V.

On the other hand, we have already reported that the absorbance of xanthene dye–iron(III) complexes is remarkably enhanced by the coexistence of substances having oxygen donor atoms, such as phosphate ion, citrate ion, organophosphorus compounds and 1,2-diphenols.[11] In addition, there are a few methods[12] for the determination of sugars by using iron(III), and Angyal and Kondo[13] have reported that sugars and polyols form complexes with metal ions in aqueous solution if they contain several hydroxy groups in a suitable steric arrangement.

From these facts, it is assumed that iron ion combines with hydrophilic sugar groups on the surface of saponin micelles, and it is deduced that saponin is a surfactant having complex-forming ability with various metal ions such as iron ion.

Application

The present method was applied to the determination of iron ion in rain water, tap water, human urine and calf serum. In this application, it was not necessary to separate iron as a hydroxide and extract iron with isoamyl alcohol. The samples were treated as follows—rain water, tap water and human urine were filtered through a membrane filter immediately after sampling and acidified with hydrochloric acid. The samples were concentrated to about 10 ml on a water-bath and used for the determination of iron ion. The calf serum was treated according to the literature.[14a] Namely, it was centrifuged after adding 7.5% trichloroacetic acid solution as a deproteinizing agent. Appropriate amounts of these test solutions were taken as required, IDA (5.0×10^{-3} M) was added as a masking agent and the iron ion content was determined according to the standard procedure. The results were compared with the data obtained by atomic absorption spectrometry (AAS), inductively coupled plasma atomic spectroscopy (ICP-AES) and the bathophen method.[14] The analytical results are shown in

Table V. Absorbance of the Qnph–Iron Solution and the Molar Ratio of [Iron(III) : Qnph] in the Presence of Carbohydrates

Carbohydrate	Absorbance of Qnph–iron solution at λ_{max}/nm			Molar ratio [Fe(III) : Qnph]
	Fe(III)	Fe(II)	nm	
(Saponin)	0.590	0.590	565	2:3
Glucuronic acid	0.575	0.575	560	1:2
Arabinose	0.478	0.425	555	2:3
Galactose	0.522	0.474	555	2:3
Rhamnose	0.556	0.550	555	2:3
Xylose	0.512	0.495	555	
Glucose	0.302	0.239	555	

Iron ion, 5.0×10^{-6} M; Qnph, 1.5×10^{-4} M; carbohydrate, 0.2%; pH, 9.3; reference, Qnph–carbohydrate solution.

TABLE VI. Determination of Iron in Rain Water, Tap Water, Human Urine and Calf Serum

Sample	Iron, amount[a] (ppb)				Recovery test[a] of proposed method	
	Proposed method	AAS	ICP–AES	Bathophen. method	Recovery[b] (%)	R.S.D.[c] (%)
Rain water	45.3	41.3	51.2		98.2	0.5
Tap water	9.8	12.0	10.1		101.2	0.6
Human urine	23.6	24.5			96.8	0.9
Calf serum	2.10×10^3			1.94×10^3	97.8	0.6

a) Mean of 5 determinations. *b)* Iron taken: 2.7 μg/10 ml. *c)* Relative standard deviation.

Table VI, and the values obtained by the proposed method were in good agreement with those obtained by the other methods.

Conclusion

An analytical application of saponin was tried by using the color reaction between Qnph and iron ion in the presence of conventional surfactants and saponin.

Saponin acted as a surfactant having complex-forming ability with metal ions such as iron ion. The sensitivity of the proposed method for the determination of iron ion (iron^{III}+iron^{II}) with Qnph and saponin is about 4—8 times that of existing methods,[8] and this method is superior to the above-mentioned methods with respect to the effect of diverse ions, the rate of color development, simplicity, and reproducibility. Application of the present method to the assay of iron ion in various samples should be feasible.

Though there are many saponins having various structural features and chemical properties, the use of saponins as surfactants having complex-forming ability with various metal ions should be effective in analytical chemistry.

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References and Notes

1) This paper is Part LXVII of a series entitled "Application of Xanthene Derivatives for Analytical Chemistry," Part LXVI. *Yakugaku Zasshi*, **107**, 640 (1987); This work was presented at the 37th Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Kobe, November 1987.

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