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A Spectrophotometric Determination of Iron(III) with ω-Aminoacetophenone-*N*, *N*-diacetic Acid Oxime*

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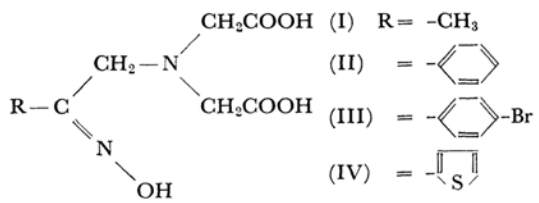
The oximes of aminoacetone-*N*, *N*-diacetic acid, ω-aminoacetophenone-*N*, *N*-diacetic acid, *p*-bromo-ω-aminoacetophenone-*N*, *N*-diacetic acid and 2-glycylthiophene-*N*, *N*-diacetic acid have been synthesized as the colorimetric reagents for iron(III). The most favourable result was obtained with the oxime of ω-aminoacetophenone-*N*, *N*-diacetic acid, which forms a stable orange red chelate with iron(III), showing an absorption maximum at 420 mμ. With the use of this reagent, 0—25 p. p. m. of iron(III) can be determined at pH 5.5. It should be noticed that this reagent is almost specific for iron(III); serious interferences were only observed for copper(II), vanadate, fluoride and phosphate ions.

During the course of our investigation of the synthesis of keto derivatives of methyliminodiacetic acid,[1] the color reactions of these ligands with various metal ions have been studied with a hope of finding new colorimetric reagents of analytical importance. In these studies, it has been found that 1-amino-propan-2-one-*N*, *N*-diacetic acid or aminoacetone-*N*, *N*-diacetic acid (I) (AADA) gave a red coloration with iron(III) in the presence of hydroxylamine hydrochloride and hydrogen peroxide; the photometric determination of iron (III) based on this color reaction has been reported.[2] The results of the detailed investigation of the mechanism of the color reaction with AADA proved that the chromogenic substance was an oxime of AADA(AADA-OX), which reacted with iron(III) to form a red chelate. This finding, together with the fact that the molar absorptivity of iron chelate of AADA-OX is not large enough (1.6×10^3), stimulated us to investigate new colorimetric reagents of the related structure.

The compounds investigated in this study are ω-aminoacetophenone-*N*, *N*-diacetic acid oxime (II) (AAPDA-OX), *p*-bromo-ω-aminoacetophenone-*N*, *N*-diacetic acid oxime (III) (Br-AAPDA-OX), and 2-glycylthiophene-*N*, *N*-diacetic acid oxime (IV) (GTDA-OX).

The comparative study of these compounds showed that AAPDA-OX and Br-AAPDA-OX



gave the most intense coloration with iron(III) with an absorption maximum in the 410—420 mμ region and that these compounds could be used as a specific colorimetric reagent for iron(III). However, AAPDA-OX may be more suitable for practical purposes because this compound can be synthesized more easily than Br-AAPDA-OX.

This paper will report on the results of an investigation of the synthesis and the use of AAPDA-OX as a colorimetric reagent for iron(III). The molar absorptivity of Fe-AAPDA-OX chelate is 2.0×10^3. This is smaller than that of Fe-1, 10-phenanthroline (1.1×10^4); however, AAPDA-OX is almost specific for iron(III). Of the common ions investigated, serious interferences were observed for only copper(II), vanadate, fluoride and phosphate ions. The results suggest that AAPDA-OX can be used as a specific iron reagent in the practical determination of iron at a relatively higher concentration level in the presence of a large amount of foreign ions. The results also indicate that a new type of colorimetric reagent for iron(III) can be developed using AAPDA-OX as a mother compound.

Experimental

The Syntheses of Reagents.—Oximes of the Keto-derivatives of iminodiacetic acid were synthesized by the condensation of the corresponding acid and the free hydroxylamine. As an example, the synthesis of AAPDA-OX(II) be described.

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1) a) T. Ando, This Bulletin **36**, 1593 (1963); b) T. Ando and K. Ueno, *Inorg. Chem.*, **4**, 375 (1965).

2) H. Takama, T. Ando and K. Ueno, *Japan Analyst*, **13**, 345 (1964).

Anhydrous sodium carbonate (1.05 g.) was added slowly to a suspension of 5 g. of ω-aminoacetophenone-*N*, *N*-diacetic acid[1b] in 15 ml. of water; this made a clear solution of the monosodium salt of the acid. To this solution was added 14 ml. of an aqueous ethanol (1 : 3 by volume) solution of free hydroxylamine prepared from 1.50 g. of hydroxylamine hydrochloride; the mixture was then heated on a water bath at 70—80°C, with occasional shaking. After the mixture had stood overnight at room temperature, the pale yellow solution was concentrated under reduced pressure almost to dryness, while the bath temperature was kept below 60°C. To the residue, 250 ml. of ethanol was added, and the solution was kept in a refrigerator overnight. The resulting monosodium salt of the oxime was filtered off, and washed with ethanol and then with ether. Yield: 7 g. The pure monosodium salt of AAPDA-OX was obtained by repeated recrystallizations from 90% ethanol. The pure oxime was obtained as colorless needles which decomposed at 129—130°C (they colored at 122°C). This material has two moles of the water of crystallization; one mole of which was lost when it was heating at 40°C over phosphorus pentoxide in vacuo, while the remaining water was removed only by heating, the material at 70°C. However, reliable analytical data corresponding to the anhydrous material could not be obtained since it was very hygroscopic.

The structure for oxime was also supported by the infrared spectra, in which a carbonyl band at 1690 cm^{-1} characteristic of the starting material disappeared in the oxime derivatives.

The physical properties and the results of analyses of the oximes are summarized in Table I. The monosodium salts of these oximes are very soluble in water and soluble in hot methanol and ethanol, but insoluble in ether, benzene, dioxane and petroleum ether. All attempts to obtain the free acid form of oximes were unsuccessful, since the oximes were easily hydrolyzed in a low pH region.

pH Titration Study.—An automatic recording titrator, Metrohm Potentiograph E 336, was employed for the pH titration study. The titration was conducted in an atmosphere of nitrogen at room temperature. A 100-ml. portion of a 1×10^{-3} M solution of monosodium salt of AAPDA-OX was titrated with a 0.1 N sodium hydroxide solution in the absence and in the presence of equimolar amounts of metal ions.

Absorption Spectral Study.—*Reagents.*—AAPDA-OX solution. A 0.001 M solution was prepared by dissolving a monosodium salt of oxime.

A 0.01 M standard iron(III) solution was prepared from ferric nitrate. This solution was standardized by EDTA titration.

The buffer solutions were acetates of standard compositions.

All the other reagents were of analytical grade unless otherwise specified.

Apparatus.—A Hitachi Model EPS-2 recording spectrophotometer was used for the measurements of the absorption spectra, while a Hitachi Model FPW-4 filter photometer was used for the photometric measurements. A Hitachi-Horiba Model P glass electrode pH meter was used for the pH measurements.

Standard procedure for the measurements of the absorption spectra: To a solution containing 312 μg. of iron(III), 5 ml. of a buffer solution (pH 5.5) and 5 ml. of a 0.001 M oxime solution were added; the mixture was then diluted to 25 ml. After 30 min., an absorption spectrum of the resulting solution was measured against water, using standard 1-cm, cells. The results are shown in Fig. 1. For the absorbance measurements, a similar procedure was followed, using a photometer equipped with a filter with a maximum transmittance at 420 mμ.

Results and Discussion

Absorption Spectra.—The absorption spectra of the iron(III) chelates of oximes and of the reagent blank are shown in Fig. 1. The reagent blank consists of iron and the buffer solution.

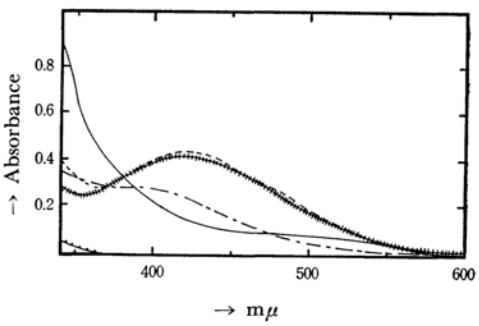

Fig. 1. Absorption spectra of oximes (12.5 p.p.m. of Fe).

- – – – AADA-OX Na
- ||||||||| AAPDA-OX Na
- – – – *p*-Br-AAPDA-OX Na
- ——— GTDA-OX Na
- ▲▲▲ Blank···Fe(III)-Acetate Buffer

None of free oxime reagents showed any absorption in the visible region. The iron(III) chelate of GTDA-OX gave no absorption maximum in this region. On the contrary, the iron(III) chelates of AAPDA-OX and Br-AAPDA-OX gave a maximum at around 420 mμ. The iron(III) chelate of AADA-OX gave a shoulder at around 390 mμ, and the absorption spectra is identical with that of the aminoacetone-*N*, *N*-diacetic acid—hydroxylamine hydrochloride — hydrogen peroxide—iron(III) system, which was reported on previously.[2] Therefore, the colored complex due to iron(III) was proved to be the iron(III) chelate of AADA-OX.

The molar absorptivity of the iron chelates of AAPDA-OX and Br-AAPDA-OX were calculated as 2.0×10^3 and 2.1×10^3 respectively. These values are not so high as 1, 10-phenanthroline, but these oximes are still effective as the colorimetric reagents for iron(III). However, the synthesis of AAPDA-OX is much easier than that of Br-AAPDA-OX. After considering these results, AAPDA-OX was chosen as a colorimetric reagent for iron(III) in this study.

Structures of the Ligand and the Iron Chelate.—As has been described in the Experimental part, the results of elementary analyses

agree fairly well with the proposed formula of the oximes. The infrared spectra of oximes, observed as Nujol mull on the solid sample as well as on the deuterium oxide solution, indicated the disappearance of the carbonyl band of the starting materials. These results all support the oxime structure.

In order to get semi-quantitative information on the interaction of various metal ions with oximes, a pH titration study was carried out on AAPDA-OX in the absence and in the presence of various metal ions. The titration curves are reproduced in Fig. 2. As is clear from the figure, a fairly strong interaction between iron(III) and AAPDA-OX can be seen in the acid region.

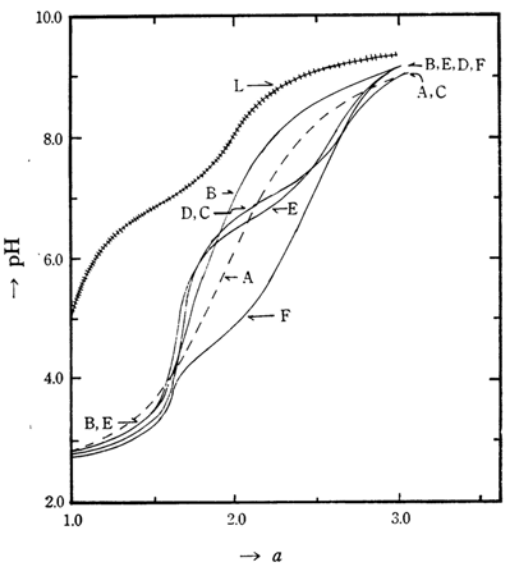

Fig. 2. Titration curves of monosodium AAPDA-OX in the abssence and presence of equivalent amount of various ions.
L, ligand; A, Fe(III); B, Cd(II); C, Zn(II); D, Ni(II); E, Co(II); F, Cu(II). *a* denotes moles of base (0.1 N NaOH) added per mole of ligand. Concentration of ligand and metal ions···about 1×10^{-3} M.

To determine the combining ratio of iron to a ligand, the continuous variation study was carried out on the Fe-AAPDA-OX system at pH 5.5. As is shown in Fig. 3, the results clearly indicate the formation of the chelate in only a 1 : 1 metal ligand ratio. Since iron(III) has a coordination number of 6, the iron(III) chelate of AAPDA-OX may possibly have a structure such as is shown in Fig. 4. Since there still remains a problem of the geometric arragement of the imino hydroxyl group (syn and anti), more experimental data is needed to settle this problem.

Conditions for the Photometric Determination of Iron(III).—In order to find the optimum conditions for the photometric determination of

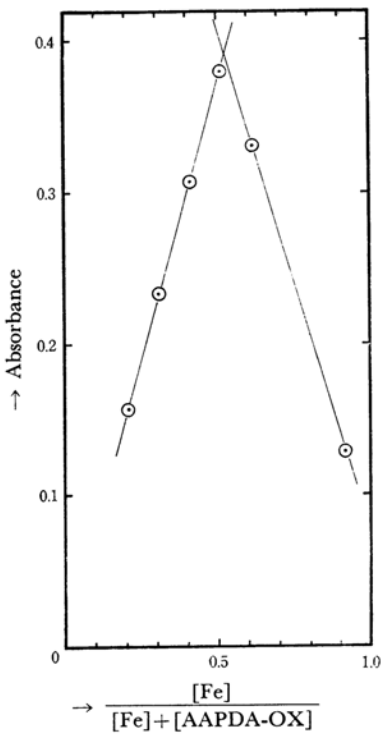

Fig. 3. Continuous variation study on AAPDA-OX-iron(III) system. [Fe(III)]+[AAPDA-OX] =0.01 M. pH=5.5. wavelength=420 mμ.

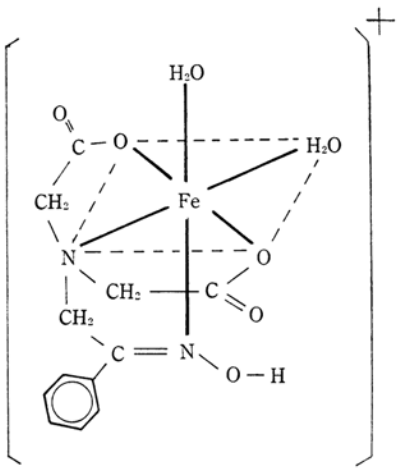

Fig. 4. Possible structure of the colored complex.

iron(III) with AAPDA-OX, various factors influencing the coloration, such as the pH, the amount of reagent, and the standing time, have been investigated.

The Effect of the pH.—The variations in the absorbance of the iron(III) chelate of AAPDA-OX at 420 mμ with a change in pH are shown in Fig. 5. The standard procedure for the measurement of absorption spectra was followed, with acetate

TABLE I. MONOSODIUM SALTS OF OXIMES

Oximes	Recrystallized from	M.p. (with decomposition), °C	Water of crystallization	Elementary analyses[a] Calculated, % C H N	Found, % C H N
I AADA-OX	85% ethanol	154—155	1 mole	for $C_7H_{11}O_5N_2Na \cdot H_2O$ 34.43 5.36 11.47	34.08 5.37 11.47
II AAPDA-OX	90% ethanol	129—130	2 moles	for $C_{12}H_{13}O_5N_2Na \cdot 2H_2O$ 44.44 5.28 8.65	44.70 5.18 8.95
III Br-AAPDA-OX	90% ethanol	134—135	2 moles	for $C_{12}H_{12}O_5N_2BrNa \cdot 2H_2O$ 35.75 4.00 6.95	36.01 3.95 7.24
IV GTDA-OX	95% ethanol	above 170 (gradually decomposed)	2 moles	for $C_{10}H_{11}O_5N_2SNa \cdot 2H_2O$ 36.36 4.54 8.48	36.23 4.55 8.24

a) Elementary analyses were carried out at the Elementary Analysis Center of Kyushu University.

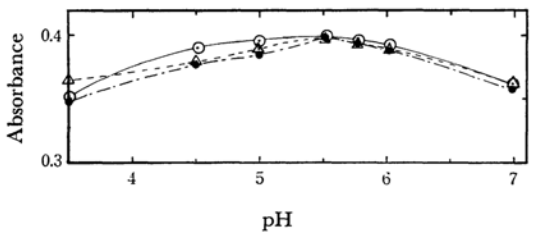

Fig. 5. Effect of pH on absorbance (12.5 p.p.m. of Fe).

●— - —● standing time 15 minutes
△- - - -△ standing time 1 hour
⊙——⊙ standing time 20 hours

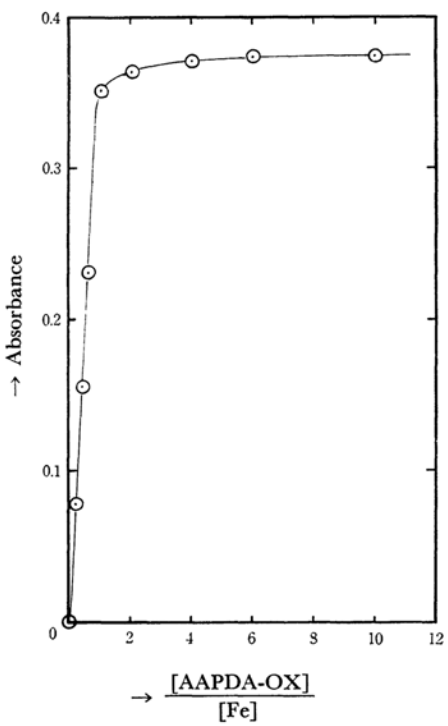

Fig. 6. Effect of the amount of reagent on the color developement (11.0 p.p.m of Fe).

buffers of different pH values, and their absorbances were measured after various standing times. It may be seen from this figure that the absorbance changes very slightly over the pH range from 4.0 to 7.0. Especially, in the pH range from 5.0 to 6.0, the absorbance is almost constant within the limits of experimental error. Therefore, the acetate buffer solution of pH 5.5 was chosen in this experiment. The amount of buffer solution had not much influence on the absorbance, since the acetato-iron(III) complex has no significant absorption at 420 mμ under these conditions.

The Amount of AAPDA-OX.—The effect of the amount of the reagent on the color development was studied. As is shown in Fig. 6, when more than four times as much reagent as iron was employed,

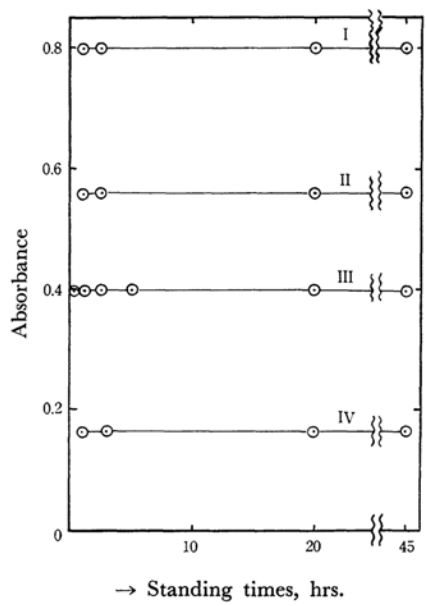

Fig. 7. Effect of the standing time for the color development.
I, Fe=25.0 p.p.m.; II, Fe=17.5 p.p.m.; III, Fe=12.5 p.p.m.; IV, Fe=7.0 p.p.m.

the absorbance became almost constant. Accordingly, 5 ml. of a 1×10^{-1} M solution of AAPDA-OX was added to a sample solution containing 50 to 625 μg. of iron(III).

The Stability of the Color.—The color developed by the standard procedure was found to be very stable over many hours, as is shown in Fig. 7. It was not neccessary to wait for the full color development, as the color reached its maximum immediately after the reagents were mixed. However, the absorbance was measured after 0.5—1.0 hr. in this experiment.

Calibration Curve.—To the series of sample solutions containing 60—600 μg. of iron(III), the standard procedure described in the experimental part was applied, and the absorbances of the resulting color at 420 mμ were plotted against their concentrations. As is shown in Fig. 8, the system follows Beer's law in the concentration range from 0 to 25 p. p. m. of iron(III).

The Effects of Diverse Ions.—The effects of diverse ions were investigated on the solutions containing 312 μg. of iron(III) and various amounts of individual diverse ions. The cations, except for manganese and chromium, were added as the nitrate, but manganese and chromium were added as sulfate and chloride respectively. The data in Table II show the tolerance limits of each ion.

As the table shows, cadmium, manganese(II), lead and ammonium do not interfere, even if they are present at a concentration level hundreds of times that of iron. Nickel, aluminum, and zinc do not interfere up to concentrations of several tens times of the amount of iron. The negative errors observed in the presence of a large amount of these interfering ions may be due to the consumption of the reagent by the formation of stable but colorless complexes with these cation; the error may be lessened by the use of a fairly large amount of the reagent. Cobalt(II) and chromium(III) show a positive error if they are present in an amount more than twice that of iron(III). Copper(II) seriously interferes, giving a positive error. The cations which give a positive error are apt

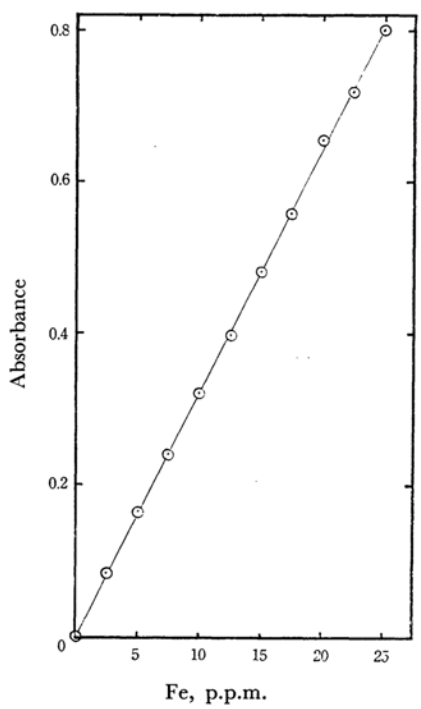

Fig. 8. Calibration curve for the determination of iron(III) with AAPDA-OX.

Table II. Effects of diverse ions for the determination of 312 μg. of iron(III)

Ions	Ions added μg.	Found Fe μg.	Relative error, %
Al(III)	3000	300	−3.85
	1500	305	−2.24
	750	310	−0.64
Cd(II)	24000	298	−4.49
	12000	315	+0.95
	6000	310	−0.64
Co(II)	600	320	+2.56
	300	318	+1.92
Cr(III)	525	318	+1.92
	263	310	−0.64
Cu(II)	625	345	+10.58
	63	318	+1.92
Mn(II)	44000	312	0.00
	22000	312	0.00
	8800	315	+0.95
Ni(II)	29500	300	−3.85
	5900	315	+0.95
	2950	310	−0.64
Pb(II)	41400	290	−7.05
	20700	315	+0.95
	10350	310	−0.64
Zn(II)	3250	295	−5.45
	1950	311	−0.32
	1300	312	0.00
NH_4^+	90000	318	+1.92
	45000	312	0.00
MoO_4^{2-}	80000	320	+2.56
	40000	315	+0.95
	8000	305	−2.24
WO_4^{2-}	4955	283	−9.29
	3300	308	−1.28
	1650	305	−2.24
VO_4^{3-}	990	360	+15.38
Cl^-	355000	320	+2.56
	177500	318	+1.92
SO_4^{2-}	480000	318	+1.92
	240000	315	+0.95
CH_3COO^-	295000	310	−0.64
	147000	315	+0.95
PO_4^{3-}	48000	103	−66.99
	950	305	−2.24
F^-	2000	278	−10.90
	200	298	−4.49

to form stable and colored complexes, and so they should be masked or separated by appropriate methods. The anions, such as chloride, sulfate and acetate, do not interfere, even if they are present at concentration levels several thousands of times that of iron. Hundreds times of the level of molybdate and tens times of the level of tungstate do not interfere either; however, as was expected for iron(III), fluoride and phosphate seriously interfere with the determination by the formation of stable fluoro- and phosphato-complexes respectively.

It is clear from these results that AAPDA-OX is almost specific for iron, and that this reagent can be used as a colorimetric reagent for iron(III) in the presence of fairly large amounts of various ins.

It is interesting to note that the degrees of interference by these cations are parallel with the degrees of chelate formation, as estimated from the titration curves in Fig. 2. The extent of the pH drop is largest for copper, intermediate for cobalt and nickel, and smallest for cadmium and zinc. Thus, the interference with the cations is caused not only by the formation of colored complexes with the reagent, but also by the formation of stable chelates even if they are colorless.

A Comparison with Other Methods. — Of the various colorimetric reagents for iron, 2, 2′-bipyridine, 1, 10-phenanthroline and 5, 8-diphenyl-1, 10-phenanthroline have been known as the most favourable reagents because of their sensitivity.[3] They form strongly-colored complexes of the hexacovalent type and chelates (FeA_3^{2+}) with iron(II) in weakly acidic, neutral, or weakly alkaline media. The complexes are fairly stable, and the reagents are highly selective for iron. However, nickel, cobalt and copper are known to form colored complexes with these reagents, giving erroneous results. The limiting amounts of the interfering ions for various reagents are compared in Table III.

As has described above, the molar absorptivity of Fe-(AAPDA-OX) is about one fifth that of Fe-(phenanthroline)$_3$. Therefore, AAPDA-OX can not compete with the phenanthroline reagents in it's sensitivity; however, as is shown in Table III, AAPDA-OX is more specific than the phenanthroline reagents for iron. As has been discussed in the previous section, by the use of this reagent iron(III) can be determined in the presence of a fairly large amount of cations, especially in the presence of nickel. It should also be noticed that AAPDA-OX reacts with iron(III) ion immediately, resulting in a maximum coloration, whereas it is necessary to wait for a while to reach a maximum coloration with phenanthroline reagents, because that color reaction involves the reduction of iron(III) to iron(II).

Table III. Limiting amounts of interfering ions for various reagents

Ion	Reagents		
	AAPDA-OX (for 12.5 p.p.m. of Fe(III)) p.p.m.	1, 10-Phenanthroline[4] (for 2 p.p.m. of Fe(III) p.p.m.	2, 2′-Bipyridine[5] for 5 p.p.m. of Fe(III) p.p.m.
Al(III)	50	0	—
Cd(II)	600	50	5
Co(II)	20	10	2
Cr(III)	20	0	1.5
Cu(II)	2	10	5
Mn(II)	2000	500	5
Ni(II)	250	2	2.5
Pb(II)	800	500	—
Zn(II)	80	10	2.5
NH_4^+	3600	500	—
MoO_4^{2-}	200	∞	—
WO_4^{2-}	150	5	3.5
VO_4^{3-}	0	0	—
Cl^-	7100	500	precipitated
SO_4^{2-}	1900	500	—
CH_3COO^-	12000	500	—
PO_4^{3-}	30	20	—
F^-	0	500	—

The result of this investigation also indicates that a new type of colorimetric reagents for iron could be developed by starting from AADA-OX or AAPDA-OX as a mother compound.

The authors wish to thank Mrs. Hiroko Yamaguchi for her laboratory assistance in the analytical work. They are also grateful for the Ministry of Education of the Japanese Government for its financial assistance.

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