Improved Sensitivity and Selectivity in the Spectrophotometric Determination of Iron by Use of a New Ferroin-Type Reagent

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THE MOST WIDELY USED TYPE of colorimetric reagent for the determination of iron is the ferroin chromogen, sometimes termed the methine chromophore. Two of the earliest and best known examples, both prepared and characterized by Blau in the late 1800's (*1*), are 1,10-phenanthroline and 2,2'-bipyridine. Literally hundreds of compounds of the same type—i.e., those that possess the —N=C—C=N— bidentate chelate functionality, have since been synthesized and investigated. Several are outstanding for their exceptional sensitivity as iron chromogens: 4,7-diphenyl-1,10-phenanthroline (Bathophenanthroline) (*2, 3*); 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) (*4, 5*); and 2,6-bis(4-phenyl-2-pyridyl)-4-phenylpyridine (Terrosite) (*6*). The molar absorptivities and wavelengths of maximum absorbance of these are, respectively, 22,400 at 533 mμ; 24,100 at 595 mμ; and 30,200 at 583 mμ. Significantly, all three of these reagents evolved from a joint program of systematic study undertaken some twenty years by G. F. Smith of the University of Illinois and F. H. Case of Temple University. The latest innovation in ferroin type reagents to come from this continuing program is the subject of this paper.

The compound 3-(4-phenyl-2-pyridyl)-5,6-diphenyl-1,2,4-triazine, hereafter abbreviated as PPDT, was among a group of compounds recently investigated for their chromogenic properties with Cu^+, Co^{+2}, and Fe^{+2} ions (*7*). It proved to be especially sensitive as an iron chromogen, imparting a molar absorptivity to its iron(II) complex of 28,700 at 561 mμ. More sensitive than TPTZ but less than Terrosite, PPDT is found to give a more stable and more extractable iron(II) complex than either. Additional studies reported here together with results obtained using PPDT for the determination of iron in sea water, beer, and milk demonstrate the analytical effectiveness of this newest of ferroin type reagents.

EXPERIMENTAL

Reagents. 3-(4-PHENYL-2-PYRIDYL)-5,6-DIPHENYL-1,2,4-TRIAZINE (PPDT), 0.005*M*. A portion of the original sample prepared by F. H. Case (*8*) was used. A solution of PPDT, 0.005*M*, was obtained by adding a few drops of hydrochloric acid to 0.193 gram of compound, followed by 100 ml of ethanol.

IRON STANDARDS. Electrolytic iron was dissolved in hydrochloric acid and diluted with distilled water to give known concentrations.

HYDROXYLAMINE HYDROCHLORIDE. A 10% solution was prepared by dissolving 100 grams of salt in 900 ml of water.

Iron was removed by adding PPDT and extracting with isoamyl alcohol.

BUFFERS. The acetate buffer was prepared to contain 1 mole each of acetic acid and sodium acetate per liter of solution. The ammonia buffer was prepared similarly using ammonium chloride and ammonium hydroxide.

MISCELLANEOUS. The isoamyl alcohol was Baker & Adamson, Purified; all other chemicals were reagent grade quality. To minimize blank corrections when wet ashing procedures are employed, the use of doubly distilled perchloric acid (G. Frederick Smith Chemical Co.) and reagent grade nitric acid that has been redistilled from an all-glass still and stored in a borosilicate glass bottle is recommended.

Apparatus. A Cary Model 14 and a Beckman Model DU spectrophotometer were used, the latter for most of the individual absorbance measurements; cells were 1-cm Corex. A Beckman Model G pH meter with glass and calomel electrodes was used for measurement of pH. Wet ashing was performed in 250-ml conical borosilicate glass flasks under a Transite hood. Some samples were also conveniently wet ashed outside of the hood using glass fume eradicators obtained from the G. Frederick Smith Chemical Co., Columbus, Ohio.

Recommended Procedure. Pipet sample of sufficient size to contain 1–15 μg of Fe into separatory funnel and add 1 ml 10% hydroxylamine hydrochloride, 2 ml of 0.005*M* PPDT, 5 ml of acetate buffer (pH 4–5), and 2 ml of $NaClO_4$ solution (50 grams per 100 m lof H_2O). Extract with 9 ml of isoamyl alcohol, and draw off the lower aqueous layer. If copper is suspected or known to be present, add 20 ml of the ammonia buffer (pH 8.5–9.2) to the contents of the separatory funnel, shake at least 15 sec, and discard the aqueous layer (which should still be alkaline; if not repeat with fresh ammonia buffer). Collect the isoamyl alcohol layer in a 10-ml volumetric flask, dilute to volume with ethanol, and measure the absorbance of the solution *vs.* a blank at 561 mμ within 30 min after the ammonia buffer treatment. Make use of a suitably prepared calibration curve or empirical equation to convert absorbance to concentration.

Study of Interferences. Various substances were tested for possible interference. The Recommended Procedure was employed to determine iron in 10-ml samples containing 1.52 μg of Fe and various amounts of added substances. The criteron for interference was a result that differed from the expected absorbance by $\pm 2\%$.

Procedure for Determination of Iron in Sea Water. Synthetic samples of sea water were prepared to contain 0–60 μg of iron, 10 mg of fluoride, and 30 grams of sodium chloride per liter. The iron content was determined using 100-ml samples and the Recommended Procedure.

Procedure for Determination of Iron in Beer. Pipet 10.00 ml of the degassed beer (if sample weight desired, measure density of a separate sample) into a 250-ml conical flask, add a 10-ml mixture of equal volumes perchloric acid (70%) and concentrated nitric acid, and heat gently until vigorous evolution of brown fumes subsides. Continue heating more strongly until dense white fumes of perchloric acid completely fill the flask and no further sign of reaction is apparent as evidenced by cessation of gaseous products evolving from within the reaction mixture (usually 2–3 minutes after fuming

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commences). Cool, add 20–30 ml of water, and boil briefly to remove any chlorine. Add 2 ml of hydroxylamine hydrochloride, 2 ml of PPDT solution, 2 ml of acetate buffer, and sufficient concentrated ammonia (approximately 4 ml) to adjust to pH 4–7 using pH indicating paper. Transfer the solution to a 60-ml separatory funnel, add 9 ml of isoamyl alcohol, shake 10–20 sec, and discard the lower aqueous phase after complete separation. Add 20 ml of the ammonia buffer, shake at least 15 sec, discard the aqueous layer, and collect the isoamyl alcohol layer in a 10-ml volumetric flask. After dilution to volume with ethanol, measure the absorbance *vs.* a blank at 561 mμ. Absorbance measurements should be completed soon (no later than 30 min) after treating the isoamyl alcohol extract with the ammonia buffer.

Procedure for Determination of Iron in Skim Milk. Pipet 5.00 ml of the skim milk (if sample weight desired, measure density of a separate sample) into a 250-ml conical flask, add a 10-ml mixture of equal volumes perchloric acid (70%) and concentrated nitric acid, and heat below the boiling temperature. [Four stages are observed during the wet ashing process: first the evolution of brown fumes of nitric oxide; then a quiescent stage during which the solution becomes increasingly more concentrated in $HClO_4$; next a rather vigorous evolution of gaseous reaction products, accompanied by dense white fumes of $HClO_4$ and lasting approximately 10 min; and finally a quiescent stage with dense white fumes completely filling the flask.] Continue heating the reaction mixture for at least 10 min after the fourth stage has been reached to completely digest the sample. After the flask and contents have cooled to room temperature, add 20–30 ml of water (washing down the sides of the flask), and boil briefly to remove any chlorine. To complete the analysis, proceed as directed above for the determination of iron in beer after the wet ashing is completed.

RESULTS

The absorption characteristics of the magenta iron(II) complex are shown in Figure 1. Beer's law is followed, the absorptivity (ϵ = 28,700) is independent of pH from 3 to 8, and color stability with time is excellent, especially at pH 4 to 6. Molar ratio and continuous variation studies identify the iron(II) species as the *tris* complex $Fe(PPDT)_3^{+2}$, with a stability constant too large to estimate from the plots which show little or no curvature in the region of the stoichiometric point.

The $Fe(PPDT)_3^{+2}$ complex is only slightly soluble in water but soluble in ethanol, ethanol-water, isoamyl alcohol, benzene, and nitrobenzene. Rate of formation of the complex from very dilute aqueous solutions of iron(II) is quite slow, because of the low water solubility of the reagent PPDT. The rate is greatly enhanced by the presence of either a soluble perchlorate salt or relatively large amounts of ethanol. The latter tends to dissolve the reagent, thereby increasing the rate of chelation. Because the perchlorate salt of the complex is extremely insoluble in water, it appears that the effect of perchlorate ions on the reaction rate is a consequence of the removal of product by precipitation. Fortunately the complex perchlorate salt dissolves readily in isoamyl alcohol. Thus the need for relatively large amounts of ethanol to favor formation of the complex, but which would make subsequent extraction into organic solvents less effective, can be avoided.

Results of various tests for interference are given in Table I. Of the ions tested only Cu^+ and Co^{+2} give colored, extractable complexes with PPDT, and these do not interfere significantly unless present in concentrations considerably greater than that of the iron. The copper(I) complex that forms and ordinarily interferes greatly undergoes pronounced color fading when its isoamyl alcohol solution is shaken with the ammonia buffer. Similar treatment of the iron(II) complex causes no imme-
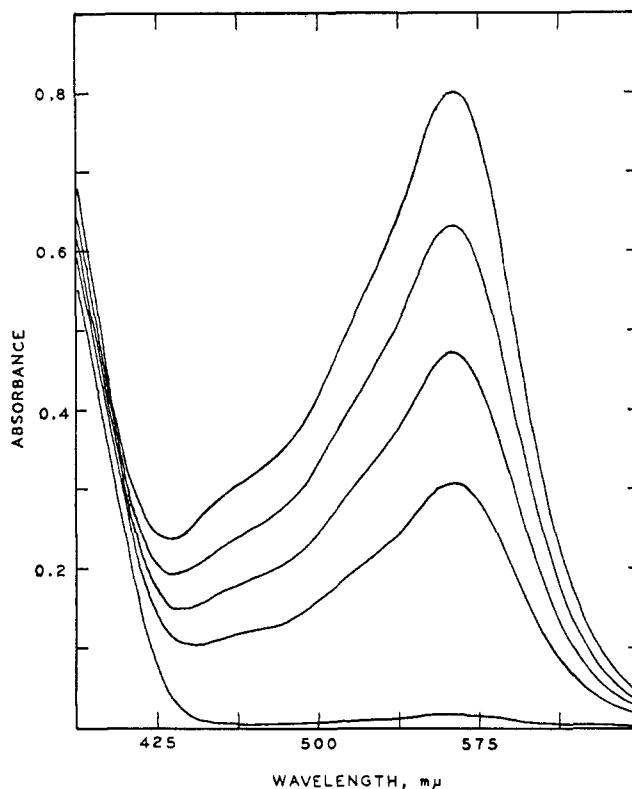

Figure 1. Absorption spectra of the magenta iron(II) complex of PPDT in aqueous ethanol solution *vs.* solvent blank

Lowest curve is spectrum of the reagent blank and four successively higher spectra are of solutions containing 0.566, 0.892, 1.190, and 1.518 ppm of Fe, respectively

Table I. Effect of Various Ions on Determination of Iron by Recommended Procedure

No interference from 1000 ppm of the following ions

Li^+, Na^+, K^+, NH_4^+, Mg^{+2}, Ca^{+2}, Sr^{+2}, Ba^{+2}, Zn^{+2}, Cd^{+2}, Ag^+, Al^{+3}, Sn^{+2}, Pb^{+2}, Th^{+4}

Cl^-, Br^-, I^-, ClO_3^-, BrO_3^-, ClO_4^-, SO_3^{-2}, SO_4^{-2}, $S_2O_3^{-2}$, $S_2O_8^{-2}$, SCN^-, NO_3^-, $C_2H_3O_2^-$, PO_4^{-3}

Interfering ions and approximate tolerance levels, ppm

Mn^{+2}	(500)	UO_2^+	(100)	Cu^+ or Cu^{+2}	(20$^+$)
MoO_4^{-2}	(500)	NO_2^{-2}	(50)	Ni^{+2}	(6$^+$)
F^-	(500)	Cr^{+3}	(30)	Co^{+2}	(4$^+$)
$C_2O_4^{-2}$	(100)	Hg^{+2}	(20)	CN^-	(<1)

Table II. Determination of Iron in Synthetic Sea Water Samples

Concentration of Fe, ppb		Error, %
Present	Found	
60.9	61.5	1.0
	61.5	1.0
24.3	24.5	0.8
	24.0	1.2
12.1	12.5	3.2
	11.5	5.0
6.09	6.0	1.5
	6.0	1.5
1.22	1.0	18.
	2.0	67.

Table III. Determination of Iron in Beer

Beer	Sample wt g	Cu added, μg	Fe added, μg	$A_{561 m\mu}$ vs. blank	Fe, μg found	Original Fe concn, ppm
Bottled	10.05	0	11.87	0.680	13.26	0.138
		0	11.87	0.682	13.30	0.142
		63	0	0.065	1.27	0.126
		63	0	0.059	1.15	0.114
		0	0	0.074	1.44	0.143
		0	0	0.068	1.33	0.132
						$\bar{X} = 0.132$
						$S = 0.011$
Canned	10.12	0	11.87	0.657	12.81	0.093
		0	11.87	0.662	12.91	0.103
		63	0	0.058	1.13	0.112
		63	0	0.060	1.17	0.116
		0	0	0.056	1.09	0.108
		0	0	0.054	1.05	0.104
						$\bar{X} = 0.106$
						$S = 0.008$
Draft	10.10	0	11.87	0.671	13.08	0.120
		63	11.87	0.673	13.12	0.124
		63	0	0.062	1.21	0.120
		0	0	0.063	1.23	0.122
		0	0	0.058	1.13	0.112
		0	0	0.063	1.23	0.122
						$\bar{X} = 0.122$
						$S = 0.005$

diate effect, although color fading occasionally is detectable after approximately 30 min. Use of scrubbing solutions more alkaline than that of the ammonia buffer will also decolorize the copper(I) complex, but the iron(II) complex also fades at a more noticeable rate. The color fading is due to oxidation of the copper(I) complex to the more nearly colorless copper (II) form by atmospheric and dissolved oxygen, catalyzed by alkaline solution. Treatment of the acetate buffered solutions with hydrogen peroxide gives similar results: the copper(I) complex fades abruptly, the iron(II) complex more slowly. If copper is known to be absent or less than 0.1 ppm, the isoamyl alcohol extract need not be treated with the ammonia buffer, and the color of the iron(II) complex remains stable for at least 1 month.

Ions that compete effectively with PPDT for complexation with iron and thus interfere are cyanide, oxalate, and nitrite ions. Most heavy metal ions can compete to some extent with iron for complexation with PPDT and thus can interfere if sufficient reagent is not provided. The tolerance levels for the various ions listed in Table I are based on the use of the amount of PPDT specified in the Recommended Procedure. Higher levels undoubtedly could be tolerated if the amount of PPDT added were to be increased.

Results of the determination of iron in sea water, beer, and milk are compiled in Tables II, III, and IV. The somewhat poor relative standard deviations (4–16%) of the replicate determinations of iron in the beer and milk samples is a consequence of the relatively low iron concentrations. Results obtained for samples with added iron exhibit much better precision (1–2% relative standard deviation), typical of that expected for spectrophotometric measurements.

Decomposition of milk and beer samples by wet ashing with nitric and perchloric acid mixtures proved more convenient than dry ashing. Temperature control is not critical and danger of iron loss by volatilization is absent. Simple tests confirmed that no iron is lost even when the nitric and perchloric acid solution is evaporated to near dryness by boiling in open beakers.

It was observed that the surest way to avoid iron-contamination from a new flask was to carry out the wet ashing procedure in it once or twice. Simple washing with hot hydrochloric acid was sometimes ineffectual.

Table IV. Determination of Iron in Skim Milk

	Concentration of Fe, ppm	
Sample	Present	Found
Milk	X	0.18
		0.14
		0.14
		0.16
		0.20
		0.20
		0.21
Milk with 12 ppm of Cu added	X	0.16
		0.20
Milk with 2.23 ppm of Fe added	$X + 2.23$	2.43
		2.41
		2.42

Mean value of $X = 0.18$ ppm
Std dev = 0.028 ppm

DISCUSSION

On the basis of its formula and spectral characteristics the $Fe(PPDT)_3{}^{+2}$ ion probably has a structure very similar to that of the *tris*(2,2'-bipyridine)iron(II) ion, with three planar bidentate ligands coordinated to the iron ion, each with two nitrogen atoms bound to iron to form a five-membered ring, and with all six nitrogens in an octahedral configuration about the central metal ion. In the case of PPDT, the two most reasonable donor atoms for steric-free chelation are the pyridyl *N*-atom and the *N*-atom in position 2 of the triazine ring. The intense absorptivity of the complex indicates that electron delocalization in the complex is pronounced, suggestive of a planar conformation for each ligand to provide electronic

conjugation among its various rings. Thus the structure of Fe(PPDT)$_3^{+2}$ may be depicted in abbreviated fashion as follows:
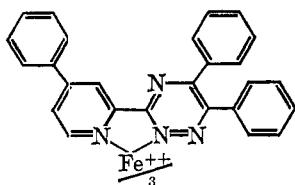
Several decided advantages are afforded by the use of PPDT for the determination of iron rather than its nearest competitor TPTZ. Extraction of the iron(II) complex of PPDT into isoamyl alcohol can be achieved quickly and quantitatively by a single extraction using as little as 9 ml of isoamyl alcohol for 100 ml or more of the aqueous phase. The molar absorptivity, color stability, and pH range of formation of the Fe(PPDT)$_3^{+2}$ complex are superior to those of the TPTZ complex. Moreover, by use of the technique whereby the color of the Cu(I) complex is destroyed on scrubbing with the ammonia buffer, considerably greater amounts of copper can be tolerated if PPDT rather than TPTZ is used. Somewhat larger amounts of Ni^{+2} and Co^{+2} are also tolerated.

In spite of its greater molar absorptivity, Terrosite is not superior to PPDT as a practical iron reagent. Some features that detract from its potential analytical value are high cost, difficulty of synthesis, low solubility in most solvents, and the rather strict solution conditions required for formation of its iron(II) complex.

By comparison with compounds that heretofore were considered to be most outstanding as spectrophotometric iron reagents, it is concluded that PPDT merits highest distinction for its sensitivity, selectivity, and ease of application.

Determination of Fluoride in Aluminum Fluoride by Wide Line Nuclear Magnetic Resonance

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THE DETERMINATION of fluoride content of aluminum fluoride is tedious and usually involves the isolation of fluoride by steam distillation from acid solution. The analysis of aluminum salts is particularly troublesome because the fluoride is so tightly bound to the aluminum. Most current methods involve loss of fluoride during the steam distillation step and errors due to variations in titration techniques. Spectrophotometric methods utilizing the blue complex formed between the fluoride ion and the cerium(III) salt of 3-amino-methyl-alizarin-N, N-acetic acid have been employed by Belcher, Leonard, and West (1). High frequency titrimetry has been used by Harley and Revinson (2) and by Calkins (3) following isolation of the fluoride by steam distillation. The advantage of high-frequency titration is a superior end point based upon changes in dielectric constant and conductivity rather than the usual visual end point based upon the bleaching effect of the fluoride ion on certain colored complexes. All of these methods, and others currently in use (4, 5), require careful technique and are nonspecific for the fluoride ion. The method presented in this paper is specific for the fluoride ion because it employs the nuclear magnetic resonance phenomenon which sees only the fluoride nucleus (6).

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EXPERIMENTAL

Apparatus. The instrument employed to determine the fluoride content of the aluminum fluoride samples was a model 104 Schlumberger NMR analyzer (now Varian PA-7). The unit has a permanent magnet of field strength equal to 1717 gauss and a homogeneity of about 1 part in 10^5 over the sample volume. The radio frequency oscillator may be switched from 7.313 Mc per second to 6.880 Mc per second in order to attain the resonance frequency of the fluoride nucleus. Distance between pole pieces is 2 inches. Provision has been made to permit variation of radiofrequency field strength step-wise over a range of 60 db. Modulation amplitude of the peak-to-peak NMR signal may be varied in ten steps in the range 0.005 to 5 gauss.

Reagents. Reagent grade sodium carbonate.

Procedure. Samples analyzed were aluminum fluoride salts which varied in purity in the range 90.00%. In the initial work NMR signals were obtained directly on the solid ore samples. A calibration curve was obtained by plotting peak-to-peak signal height as a function of the amount of aluminum fluoride present in an inert solid powder of similar density but containing no fluoride. Linear plots were obtained which were acceptable for analyses of aluminum fluoride ores to a precision of a few per cent. However, for more precise results it was necessary to fuse the aluminum fluoride ore samples in reagent grade sodium carbonate and place the resultant sodium fluoride salts in aqueous solution prior to NMR measurement. The following procedure was adopted. Samples of aluminum fluoride ore in the range 800 to 600 mg were fused with excess sodium carbonate in a closed platinum crucible in a muffle furnace or over a Meker burner. Molten mass was allowed to cool to room temperature and then the crucible plus contents were placed in a beaker