Chelatometric Determination of Ferrous Iron with 2-Pyridinealdoxime as an Indicator

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Ferrous iron may be quantitatively determined by titration into a known EDTA solution containing 2-pyridinealdoxime as an indicator. The principal advantages of this method over oxidation-reduction procedures are that the reagents are more stable and reducing substances, in general, do not interfere.

CLASSIC METHODS for the quantitative determination of ferrous iron have usually involved oxidation-reduction titrations. In these procedures, reducing substances tend to interfere, and the titrating solutions are often unstable, requiring frequent standardizations. A spectrophotometric approach, utilizing the 2-pyridine ketoxime-ferrous chelate, has been proposed by Banerjea and Tripathi (1); however, this type of assay has the disadvantages of being cumbersome and/or requiring special equipment. Because of the advantages of convenience, rapidity, and specificity claimed for chelatometric titrations, much recent work has been reported on this approach to the analysis of metallic ions (2). Notably lacking in the literature, however, is a simple titrimetric chelatometric procedure for determining ferrous iron. The present report describes a titrimetric assay for ferrous iron with 2-pyridinealdoxime (2-PA) as an indicator.

Solutions of ferrous iron form a deep red-colored chelate with 2-PA (3) which is easily detected at low concentrations. A solution of unknown concentration of iron is titrated into a solution of known concentration of EDTA containing 2-PA. The iron first combines stoichiometrically with the EDTA, and the subsequent formation of the red color of the Fe^{++}-2-PA chelate denotes the end point.

This assay method was applied to several official and commercial preparations containing ferrous salts and the results compared favorably with the official assays and/or label-stated quantities.

METHOD

Reagents.—2-Pyridinealdoxime, m.p. 111–113°; indicator solution consisting of a 0.1% solution of 2-PA; EDTA, analytical reagent grade were employed. The known solution of EDTA was prepared by dissolving 4.380 Gm. of EDTA with the aid of three equivalents of NaOH per equivalent of EDTA in enough distilled water to make 500 ml., to yield a solution of approximately 0.03 *M* (disodium EDTA also may be used). This solution was standardized against a known ferrous solution prepared from analytical grade iron wire; 0.50 *M*, pH 5 acetate buffer; salicylic acid, citric acid, and ascorbic acid, all U.S.P. grade; iron wire, analytical reagent grade. All other salts were analytical reagent grade.

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TABLE I.—ASSAY OF FERROUS AMMONIUM SULFATE SOLUTIONS

Assay with Ceric Nitrate, Molar Ferrous	Assay with EDTA and 2-PA, Molar Ferrous	Recovery, %
0.0150	0.0151	100.7
0.00750	0.0076	101.3
0.00375	0.0037	99
0.0600	0.5975	99.6

TABLE II.—TEMPERATURE EFFECTS ON THE ASSAY OF FERROUS AMMONIUM SULFATE SOLUTIONS

Approximate Temp., °C.	Ferrous Theoretical, *M*	Ferrous Observed, *M*	Recovery, %
25	0.0300	0.0310	103.3
35	0.0300	0.0306	102
50	0.0300	0.0301	100.3
65	0.0300	0.0300	100
70	0.0300	0.0300	100
85	0.0300	0.0297	99

Procedure.—An appropriate sample of the ferrous salt or the ferrous containing dosage form was dissolved in distilled water, filtered if necessary, and made to volume in a volumetric flask. For best results the final concentration of iron was found to be approximately 0.03 *M*. This solution was the titrant. Five milliliters of pH 5 acetate buffer were added to a mixture of 1 ml. of 2-PA indicator solution and 2–4 ml. of the known EDTA solution. This mixture was heated to approximately 50–70° and titrated immediately with the iron solution. The solution turned from a pale yellow-green to pink at the end point. The reaction was stoichiometric; the iron consumed was equivalent to the EDTA

$$\text{ml.}_{\text{EDTA}} \times M_{\text{EDTA}} = \text{ml.}_{\text{iron}} \times M_{\text{iron}}$$

The results of this method were compared to results obtained by titration with ceric nitrate and are shown in Table I. These and subsequently reported values are an average of at least three determinations.

Temperature Effect.—The results of the effect of temperature on the appearance of the end point are shown in Table II. Below 50° the end point appeared prematurely, probably because the Fe^{++}-2-PA chelate once formed is not easily dissociated

TABLE III.—VOLUME EFFECTS ON THE ASSAY OF FERROUS ION

Approx. Total Soln. Vol. at End Pt.	Indicator Vol.	Ferrous Theoretical, *M*	Ferrous Observed, *M*
12	2	0.0150	0.0153
12	1/2	0.150	0.0148
12	1	0.0300	0.0301
17	1	0.0300	0.0299
22	1	0.0300	0.0294
22	2	0.0300	0.0298
32	2	0.0300	0.0296
32	1	0.0300	gradual end pt.

at the pH of the assay. It was extremely difficult to eliminate the red color of the chelate, even in the presence of EDTA. The application of heat apparently hastened the equilibrium between the iron and the chelating agents.

pH Effect.—At a pH of 4.5 or below, the end point was gradual and indistinct. At pH's greater than 6, the avidity of the ferrous ion for 2-PA was great enough to produce its characteristic color before the theoretical end point. A satisfactory end point was produced in the range of approximately pH 4.5 to 5.5.

Volume Effect.—Known amounts of distilled water were added to the titration vessel to determine the effect of increased volume on the end point. The results shown in Table III indicate that the end point was slightly delayed at large solution volumes. This observation suggests that the pink color produced at the end point cannot be immediately detected in dilute solutions. In titrations where the total volume of solution at the end point was 22 ml. or more, the addition of an extra milliliter of indicator yielded good results. The effect of indicator volume on the titration was similar to the dilution effect (see Table III). Too little indicator delayed detection of the end point, while too much favored production of the Fe^{++}-2-PA complex with a resultant premature end point.

TABLE IV.—ASSAY OF FERROUS IN THE PRESENCE OF FERRIC ION

Ferric Determined by EDTA-Salicylic Acid Titration, *M*	Total Iron by EDTA-2PA Titration, *M*	Ferrous, *M* (Col. 3 less Col. 2)	Ferrous Theoretical, *M*	Ferric Theoretical, *M*
0.0210	0.0312	0.0102	0.0100	0.0207
0.0105	0.03065	0.02015	0.0200	0.0103
0.0056	0.03055	0.0250	0.0250	0.0052
0.0022	0.0306	0.0284	0.0281	0.0019

INTERFERING SUBSTANCES

Metal Ion Interference.—Metal ions which have high chelate stability constants with EDTA interfered with the assay by preferentially binding the EDTA. The following ions interfered: Mn^{++}, Zn^{++}, Cd^{++}, Ni^{++}, Cu^{++}, Co^{++}, and Fe^{3+} (see below). In the presence of 0.03 *M* Fe^{++}, Ca^{++} interfered above 0.05 *M*. Two molar KNO_3, 3 *M* NaCl, and 1 *M* $MgSO_4$ did not interfere.

Calcium Interference.—Since calcium salts are often ingredients in iron-containing dosage forms, an effort was made to analyze ferrous ion in the presence of calcium. An excess of Na_2SO_4 was added to a known Fe^{++}-Ca^{++} solution; the solution was filtered and made up to volume. Analysis showed that this procedure yielded accurate results for 0.03 *M* Fe^{++} solutions in the presence of at least 0.80 *M* Ca^{++}.

Ferric Interference.—Although Fe^{3+} interfered, the assay gave accurate results of total iron present up to at least a 3:2 ratio of Fe^{3+} to Fe^{++}. A chelatometric procedure based on that suggested by Cheng, *et al.* (4), in combination with the present assay, could be used to determine Fe^{++} in the presence of Fe^{3+}. Two to four milliliters of an approxi-

TABLE V.—ASSAY OF DOSAGE FORMS

Dosage Form	Total Iron, 2-PA-EDTA Tit.	Ferric, Sal. acid-EDTA Tit.	Other Assay Method
Ferrous sulfate syrup U.S.P.	4.12 Gm./100 ml.	negligible	N.F., 4.08 Gm./100 ml.
Ferrous iodide syrup U.S.P.	67 mg./ml.	negligible	N.F., 67.0 mg./ml.
Ferrous gluconate capsules, 325 mg.	332 mg.	13 mg.	U.S.P., 338 mg.
Ferrous lactate drops, 25 mg./ml.	26.9 mg./ml.	uncertain	Ceric, no good
Iron-amino acid complex, 40 mg./ml.	40.9 mg./ml.	uncertain	Ceric[a], 42.4 mg./ml.
Vitamin-iron capsules[b] Ferrous sulfate, 160–200 mg.	167 mg.	uncertain	...
Vitamin-iron tablets$_1$[b] Ferrous sulfate, 300 mg.	310.3 mg.	uncertain	...
Tablets$_2$[b], Ferrous lactate, 30 mg.	31.6 mg.	20 mg.[c]	...
Prepared mixture[b] Ferrous sulfate, 300 mg.	297.6 mg.	uncertain	...

[a] This high value is probably because of unknown additives in the preparation.
[b] Composition of vitamin products:

	Capsules	Tablets$_1$	Tablets$_2$	Prepared Mixture
Desicated liver	325 mg.	100 mg	...	350 mg.
B_{12}	1.7 mcg.	1/3 U.S.P. u.	...	1 mcg.
B_1	2 mg.	2 mg.	...	10 mg.
B_2	2 mg.	2 mg.	...	10 mg.
B_6	1 mg.	...	...	5 mg.
Niacin	10 mg.	10 mg.	...	50 mg.
Folic acid	0.4 mg.	2 mg.	...	3 mg.
C	50 mg.	50 mg.	...	150 mg.
Pantothenic acid	2 mg.	...	...	25 mg.
Calcium lactate	...	...	350 mg.	...
A	...	...	1500 U.S.P. u.	...
D	...	...	150 U.S.P. u.	...
$Al(OH)_3$	...	...	150 mg.	...
Ethyl amino benzoate	...	...	35 mg.	...

[c] These tablets were at least 5 years old.

mately 0.03 *M* iron solution in 0.01 *N* HCl is titrated with EDTA using salicylic acid as an indicator. The disappearance of the red-violet (Fe^{3+}-salicylic acid chelate) color denotes the end point. At this low pH, Fe^{++} combines to a very slight degree with EDTA and the assay gives only Fe^{3+} concentration. The concentration of total iron present and of Fe^{3+} can be used to determine ferrous ion by difference. If the unknown solution has a color of its own, this method fails because the end point in the Fe^{3+} titration cannot be accurately observed. Results of this method are shown in Table IV.

Chelating Agent Interference.—Two chelating agents often found in iron preparations, which might interfere with the assay by binding ferrous ion, are citric acid and ascorbic acid. In known samples containing 0.03 *M* Fe^{++}, citric acid interfered when present in concentrations greater than 0.03 *M*. Ascorbic acid did not affect the assay in concentrations up to at least 0.50 *M*.

ASSAY OF COMMERCIAL PREPARATIONS

Several U.S.P. and commercially available products were assayed for iron content to test the applicability of the method to more complex preparations. Liquid dosage forms were appropriately diluted so that the final concentration of iron was approximately 0.03 *M*. Solid dose forms were prepared by filtering an aqueous suspension of pulverized tablets or capsule contents through a sintered-glass funnel and making up to volume in a volumetric flask. If calcium was present, sodium sulfate was added prior to filtration, as previously described.

Since no other assay method could be conveniently used, the analysis of commercial preparations by this method could only be compared to the labeled amount of iron. Therefore, a mixture was prepared from vitamin capsules and folic acid tablets to contain a known quantity of ferrous sulfate; the assay showed good recovery for ferrous ion (see Table V).

Whenever possible, known assay methods were used to verify the results of the chelatometric titration. Although the commercial liquid iron preparations tested contained only a single active ingredient, titration with ceric nitrate failed, perhaps because of interfering additives. Also the presence of ascorbic acid in other preparations precluded the use of ceric nitrate.

Although some of the diluted iron solutions were colored, the end point of the 2-PA titration was readily distinguishable. However, ferric ion could not be determined in these same preparations because the end point with salicylic acid at pH 2 is not sharp enough to be observed when interfering colors are present.

Results of these assays are shown in Table V.

SUMMARY

When compared to oxidation-reduction and other official methods for the determination of ferrous ion the titrimetric chelatometric assay described in this report presents a more advantageous method under certain conditions: (*a*) Reducing substances, which interfere in oxidation-reduction methods, usually become innocuous in the chelatometric titration. These include such substances as ascorbic acid and possibly certain fillers and flavors in dosage forms. In the case of ferrous iodide syrup N.F., this assay gave accurate results, which compared favorably to the N.F. method and which obviated much of the tedium associated with the official assay. (*b*) The solutions used in the chelatometric titration are extremely stable. In addition, EDTA can be purchased in a virtually 100% pure state; standardized EDTA solutions corresponded very closely to the amount of EDTA used to prepare these solutions. (*c*) Total iron content may be determined even in the presence of large amounts of ferric ion.

Oxidation-reduction methods are more efficacious when ferrous is to be determined in the presence of ferric ion. Also, certain chelating metals and chelating agents interfere with the chelatometric titration. In these cases, oxidation-reduction assay methods can be used to advantage.

Under certain conditions, the procedure for ferrous determination described in this report is advantageous compared to established methods and presents an approach which may be utilized where other methods fail.

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