and proposed methods was 97.4 and 96.7%, respectively, for undegraded aspirin and 42.9 and 35.2%, respectively, for aspirin subjected to stress conditions. The comparison is satisfactory, especially if the method is used for screening purposes in formulation work.

REFERENCES

(1) "The National Formulary," 13th ed. Mack Publishing Co., Easton, Pa., 1970, p. 66.

(2) K. T. Koshy, *J. Pharm. Sci.*, **53**, 1280 (1964).

(3) J. Levine and J. D. Weber, *ibid.*, **57**, 631 (1968).

(4) C. A. Kelly, *ibid.*, **59**, 1053 (1970).

(5) B. Gehauf, J. Epstein, G. B. Wilson, B. Witten, S. Sass, V. E. Bauer, and W. H. C. Rueggeberg, *Anal. Chem.*, **29**, 278 (1957).

(6) J. S. Hanker, A. Gelberg, and B. Witten, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 728 (1958).

(7) R. C. Garner, A. L. Wallpole, and F. L. Rose, *Cancer Lett.*, **1**, 39 (1975).

(8) D. J. Marsh and E. Neale, *J. Appl. Chem.*, **8**, 394 (June 1958).

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Single-Dose Assay of Ferrous Ion in Pharmaceuticals

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Abstract □ A method for the assay of the ferrous ion in hematinics or multivitamin tablets is described. The method is based on the spectrophotometric measurement of the chromophore produced by reacting the ferrous ion with the highly specific chelating agent ferrozine. The method is proposed as an alternative to the redox methods in the USP and NF.

Keyphrases □ Ferrous ion—spectrophotometric analysis, pharmaceutical formulations □ Iron—ferrous ion, spectrophotometric analysis, pharmaceutical formulations □ Spectrophotometry—analysis, ferrous ion in pharmaceutical formulations □ Hematinics—ferrous ion, spectrophotometric analysis in pharmaceutical formulations

The presence of the ferrous ion in many pharmaceutical preparations necessitates a rapid, economical, and sensitive method for its determination in pharmaceuticals. The official USP and NF methods primarily rely on the classical redox conversion of the ferrous to the ferric ion (1, 2). These redox methods are tedious and inaccurate due to difficult end-point visualization.

Other methods are available for the assay of iron-containing products, *e.g.*, atomic absorption spectrophotometry and X-ray emission spectrometry. However, these methods require involved sample preparation as well as sophisticated instrumentation (3, 4). This report describes a simple colorimetric procedure applicable to ferrous-containing pharmaceuticals such as hematinics and multiple-vitamin preparations with iron. The sensitivity is such that the method is ideal for single-dose assays.

The present method utilizes the reagent ferrozine, 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine disodium salt, a chelating agent highly specific for the ferrous ion. Although this compound is similar to the well-known chelating agent 2,4,6-tripyridyltriazine, previously used for iron assays of pharmaceuticals, it has the advantages of being significantly more water soluble, more sensitive, and possibly more economically feasible for repeated analyses (4, 5).

Ferrozine has been shown to react with the ferrous ion to yield a magenta-colored tris complex, *i.e.*, Fe-(ferrozine)$_3^{2+}$. This species forms completely in aqueous solution between pH 4 and 9 and exhibits a sharp peak with maximum absorbance at 562 nm (5). Interference studies showed that only monovalent copper and divalent cobalt formed colored species with ferrozine (5). Ferrozine was used in the determination of iron in municipal waters (5) and serum iron levels (6).

Table I—Stability of Iron–Ferrozine Complex

Hours after Mixing	Absorbance[a]	Absorbance[b]	Absorbance[c]
0.17	0.492	0.615	0.520
0.50	0.492	0.615	0.520
1.00	0.492	0.615	0.520
2.00	0.492	0.615	0.520
8.00	0.496	0.615	0.518
24.00	0.496	0.611	0.518
48.00	0.496	0.611	0.511

[a] Obtained from assay of ferrous fumarate tablet (Feostat, Westerfield Co.). [b] Obtained from assay of ferrous sulfate tablet (Feosol, Smith Kline and French). [c] Obtained from assay of analytical grade ferrous ammonium sulfate.

EXPERIMENTAL

Instrumentation—A double-beam spectrophotometer[1] and a pH meter[2] were used.

Reagents and Chemicals—The following ACS grade chemicals were used: sodium phosphate monohydrate, ascorbic acid, ferrous ammonium sulfate hexahydrate, acetic acid, and ferrozine[3]. Deionized water was obtained from a commercial deionizer[4] and was shown by conductance to contain not more than 0.25 ppm of dissolved ionic solids.

Color Reagent—Ferrozine, 125 mg, was placed in a 25-ml volumetric flask, and deionized water was added to volume. The solution should be protected from light.

Buffer Solution—Sodium phosphate, 6.25 g, and 12 mg of ascorbic acid were dissolved in 100 ml of deionized water. The pH of the resultant solution was adjusted to 5.3 by the dropwise addition of 10% sodium hydroxide.

Stock Solution of Ferrous Ammonium Sulfate—Ferrous ammonium sulfate, 615 mg, was placed in a 100-ml volumetric flask and brought to volume with deionized water.

Working Solution of Ferrous Ammonium Sulfate—For the working

[1] Beckman model 25 spectrophotometer.
[2] Coleman Metrion IV.
[3] Nutritional Biochemical Corp.
[4] Culligan deionizer.

Table II—Assay Results of a Single Ferrous Sulfate Tablet[a]

Replication	Amount Found, mg	Percent Label
1	62.4	96.0
2	62.9	96.7
3	62.5	96.2
4	62.4	96.0

[a] Feosol tablets.

solution, 2.0 ml of the stock solution was placed in a 100-ml volumetric flask and brought to volume with deionized water.

Calibration Curve—Into separate 50-ml volumetric flasks were added 5.0, 4.0, 3.0, 2.0, or 1.0 ml of ferrous ammonium sulfate working solution, 2.0 ml of buffer solution, and 25 ml of deionized water. To these mixtures was added 1.0 ml of color reagent, and the mixtures were then brought to volume with deionized water. The solutions were allowed to stand with intermittent shaking for a minimum of 10 min, after which the solutions were read spectrophotometrically at 562 nm against a blank containing 2.0 ml of buffer solution and 1.0 ml of color reagent. (*Note:* Blanks did not give any visible color change upon addition of color reagent.)

Sample Preparation—*Solid Samples*—The tablet or contents of one capsule was placed in 100 ml of boiling deionized water containing 5 mg of ascorbic acid, and the mixture was boiled until complete dissolution occurred. The boiling mixture was then quantitatively filtered through a filter paper previously rinsed with several portions of boiling deionized water. After filtration, the residue on the filter paper was washed once with 50 ml of boiling deionized water followed by 100 ml of deionized water at room temperature. (If the sample contains ferrous fumarate, 100 ml of 0.1 *N* HCl should be used as the dissolution solvent.)

The mixture was then brought to volume. The final volume is variable and dependent on the labeled amount of iron. For example, if it is labeled 65 mg of elemental iron, the final volume may be 1 liter; if 33 mg of iron, then 500 ml; *etc.* One milliliter of this solution was then placed in a 50-ml volumetric flask, and the sample was prepared as were the solutions for the calibration curve and read against a blank prepared as described.

Liquid Samples—The liquid preparation (1.0 ml) was placed in a suitable volumetric flask (1 liter, 500 ml, *etc.*) and brought to volume with deionized water. Then 1.0 ml of this solution was placed in a 50-ml volumetric flask, and the sample was prepared as already described.

RESULTS AND DISCUSSION

A plot of absorbance *versus* concentration demonstrated that the Beer–Lambert law was obeyed over a wide concentration range as previously observed (5). Previous work indicated that an incubation step was needed for full color development (5, 6), but this step was shown to be unnecessary in these laboratories. Each calibration point of the Beer–Lambert plot was composed of three determinations. Two of these determinations utilized a 10-min incubation period at 40°; the third determination was allowed to stand at room temperature for this same period. Reading all three samples at room temperature gave readings that did not differ significantly, *i.e.*, a typical average deviation of 0.006 absorbance unit. Thus, it was only necessary to wait 10 min after mixing to obtain absorbance readings.

Once the color formed, it was stable almost indefinitely if kept at room temperature. Table I demonstrates the permanency of the chromophore produced. The data suggest that the permanency of color was not affected by product formulation, since iron from two different commercial formulations yielded colors as permanent as that obtained from analytical grade ferrous ammonium sulfate. The excellent stability of the chromophore offers distinct advantages when large numbers of assays must be performed since the chromophore can be produced and assayed at some later time without concern for stability. The instant and permanent color development was probably due to the fact that the concentration of ferrozine in the color reagent was well in excess of the required stoichiometric quantity.

Table III—Assay Results of a Single Multivitamin Tablet Containing Ferrous Fumarate[a]

Replication	Amount Found, mg	Percent Label
1	99.4	99.4
2	100.4	100.4
3	98.8	98.8
4	99.4	99.4

[a] Tabron.

Table IV—Assay Results Obtained from Synthetic Multiple-Tablet Composite Containing 33.04 mg of Iron per Tablet[a]

Sample	Iron per Tablet, mg	Recovery, %
1	33.31	100.8
2	33.10	100.2
3	33.25	100.6
4	33.31	100.8
5	33.21	100.5
6	33.35	100.9
7	33.25	100.6
8	33.15	100.3
9	33.15	100.3
10	33.20	100.5

[a] Prepared from ferrous ammonium sulfate and Clusivol tablets (Ayerst).

Tables II and III give typical results of replicated assays on a single tablet and demonstrate that the method gives values of relatively high precision. Initially, values were low for solid preparations containing ferrous fumarate. However, use of 0.1 *N* HCl for dissolution promoted the quantitative dissolution of the poorly soluble ferrous fumarate. The quantitativeness of the reaction with iron in pharmaceutical dosage forms was assured by adding ferrous ammonium sulfate to several iron-free multivitamin preparations of the solid or liquid type. In all cases, the iron content of these prepared samples was no less than 98.5% nor more than 101% of the amount added.

For example, the data in Table IV were obtained from a synthetic multiple-tablet composite. The composite was prepared by adding an accurately known amount of ferrous ammonium sulfate to 20 iron-free multivitamin tablets whose weight also was accurately known. The mixture was then thoroughly triturated, and approximately one-twentieth of the combined weight was used as a tablet sample. The data indicate that recovery and precision were excellent. It is assumed that the formulation characteristics of the spiked samples were comparable to the iron preparations assayed. This assumption appears to be valid, since assay of the iron-containing commercial products led to values very close to label claims.

Thus, the proposed method seems to offer several advantages over the present USP–NF method in terms of time, sensitivity, and convenience, especially when it is realized that one reagent suffices for all ferrous-containing products and that there are no interferences with tablet fillers, excipients, or colorants. The procedure should also have utility in automated assay methods.

REFERENCES

(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 196.

(2) "The National Formulary," 14th ed., Mack Publishing Co., Easton, Pa., 1975, p. 280.

(3) H. I. Tarlin and M. Batcholder, *J. Pharm. Sci.*, **59**, 1328 (1970).

(4) W. F. Beyer and K. G. Zipple, *ibid.*, **57**, 653 (1968).

(5) L. L. Stookley, *Anal. Chem.*, **42**, 779 (1970).

(6) J. P. Persijn, W. Van Der Slik, and A. Riethorst, *Clin. Chem. Acta*, **35**, 91 (1971).

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