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Determination of Ferrous Iron in the Presence of Ferric Iron With Bathophenanthroline

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ACCORDING to the tenth edition of *Standard Methods*,[1] "Probably no chemical determination involves more uncertainty than that of iron." Although the colorimetric methods applicable to natural waters are relatively satisfactory for the determination of total iron, difficulties may be encountered in the specific determination of ferrous iron. The analytic method recommended for the specific determination of ferrous iron in the ninth edition of *Standard Methods*[2] and referred to in the tenth edition * has been found, as part of a quantitative investigation on the kinetics of ferrous iron oxidation by dissolved oxygen, to lack specificity for the determination of trace (less than 1 mg/l) quantities of ferrous iron in the presence of ferric iron.

Some of the difficulties encountered in making an analysis for ferrous iron with the use of the recommended 1,10-phenanthroline method,[1] and a method using bathophenanthroline, 4,7-diphenyl-1,10-phenanthroline for the specific determination of ferrous iron, will be discussed here.

* Authors' comment on the revised phenanthroline method included in the eleventh edition of *Standard Methods*[2a] (which appeared subsequent to the preparation of the present article) will be published in the "Notes and Comment" section of a forthcoming issue of the JOURNAL.—ED.

1,10-Phenanthroline Method

The 1,10-phenanthroline method for the determination of total iron is based on the reduction of the iron to the ferrous state and its subsequent reaction with 1,10-phenanthroline to form a highly colored stable complex suitable for spectrophotometric analytic procedures.[1] It has been generally assumed that ferrous iron may be determined by merely omitting the reducing agent in the 1,10-phenanthroline procedure for the determination of total iron.[1, 2] Harvey, Smart, and Amis[3] reported that ferrous iron and the total iron concentration can be determined on a single sample by measurement of the absorbance of the ferrous- and ferric-1,10-phenanthroline complexes at two different wavelengths. But investigations by the present authors have shown that both the procedure in *Standard Methods* and that by Harvey and associates are applicable only under certain conditions. The conditions necessary to obtain reliable results are: (1) 1 mg/l or more of ferrous iron must be present in the solution being analyzed, (2) the molar ratio of 1,10-phenanthroline to total iron (ferrous plus ferric) must exceed 30, (3) the absorbance measurements must be made within 10–15 min after color forma-

tion, and (4) the solution must be free of interfering materials and protected from direct sunlight.

When the sample contains less than approximately 1 mg/l ferrous iron in the presence of ferric iron, the color formed is unstable and increases with time. For example, a 40–60 per cent increase in absorbance was observed for various ferrous-ferric mixtures in a period of 30 min. The increase in color with time is the result of the reduction of trivalent iron to ferrous-1,10-phenanthroline. This reduction can be attributed to the larger stability constant of ferrous-1,10-phenanthroline complex, as compared to the ferric-1,10-phenanthroline complex.[4, 5] The mechanism of this reduction is under investigation.

The second condition of a 30-fold molar excess of 1,10-phenanthroline to the total iron must be met to obtain stable color development in the optimum range for this procedure. If a ratio of less magnitude is used, the developed color changes rapidly with time. The large excess is based on total iron, as both ferrous and ferric iron form complexes with 1,10-phenanthroline.

In addition to the formation of complexes with bivalent and trivalent iron, phenanthroline forms complexes with many other metals. Smith and Richter [6] reported that the bivalent ions of cadmium, copper, zinc, nickel, chromium, and ruthenium also form complexes with phenanthrolines. These metals form either colored or noncolored complexes dependent on the particular metal. Because phenanthroline is a base, it combines with a proton to form phenanthrolium ion; therefore, the amount of excess phenanthroline necessary is dependent on pH. At a pH of less than approximately 5, the protons in solution compete with the iron for the available positions on the nitrogen atoms of 1,10-phenanthroline. Therefore, the amount of excess phenanthroline is dependent on the acidity of color formation, particularly at pH values less than 4–5.

It should be pointed out that in the determination of the total iron content of industrial wastes, which contain a large concentration of other metals, a larger than normal excess of 1,10-phenanthroline may be needed to overcome the amount of 1,10-phenanthroline complexed by other metals; otherwise low readings may be obtained because of an insufficient excess of 1,10-phenanthroline.[7, 8] Even under the optimum condition, the color developed from ferrous-1,10-phenanthroline is not stable over long periods of time. The color developed under the optimum condition from a mixed ferrous-ferric solution increases especially rapidly with time if the sample is allowed to stand in direct sunlight.

Because the 1,10-phenanthroline method for the determination of ferrous iron was not generally applicable to the conditions that would be encountered in the authors' investigation of the kinetics of oxygenation of ferrous iron, and to those conditions that may be encountered in the analysis of the ferrous iron content of surface and ground waters, it was necessary to review the analytic methods that are reported to be specific for ferrous iron.

Previously Reported Methods

Numerous volumetric titration procedures using oxidizing agents (MnO_4^-, $Cr_2O_7^{--}$, Ce^{4+}) have proved to be of value for the determination of relatively large quantities of ferrous iron in the absence of other reducing agents.

These methods, however, are not applicable to the determination of ferrous iron at the low concentration generally found in natural waters.[9]

Another procedure that is used for analytic differentiation of ferrous iron from ferric iron is polarography.[10] But as with the volumetric titration procedures, polarography is limited to use with relatively large concentrations of ferrous iron, compared to those normally found in surface waters. The coulometric titration procedure described by Cooke and associates[11] is an extremely sensitive method for ferrous iron determination, but it requires the use of instrumentation not normally present at water plants.

Because of their sensitivity and ease in performing an analysis, colorimetric procedures have been the most widely used methods for the determination of ferrous iron in natural waters. A variety of color-developing reagents have been used, ranging from thiocyanate, which stabilizes the ferric iron state, to the pyridyls,[12] nitrosophenol,[13] and several derivatives of phenanthroline, all of which stabilize the ferrous iron state to form highly colored compounds.[6] These reagents, however, are capable of disturbing the ferrous-ferric equilibrium. Some difficulties have been reported concerning the use of these reagents for specific ferrous iron determination. For example, Hutchinson[14] reported that the thiocyanate and dipyridyl methods for the determination of ferrous iron in natural waters do not give reliable results. The procedure using nitrosophenol as the color-developing agent is reported to have a greater sensitivity for ferrous iron than do the pyridyls,[13] but this procedure requires rigid control of experimental conditions to obtain reliable results.

Analytic methods using 1,10-phenanthroline or one of its substituted derivatives as the chromatogenic agent have been widely employed for the determination of total iron of natural or treated waters[15]; 1,10-phenanthroline has been suggested for specific determination of ferrous iron.[1,3] But, as has been previously pointed out, 1,10-phenanthroline has limited applicability for the specific determination of ferrous iron in the presence of ferric iron.

As the 1,10-phenanthroline procedure did not yield reliable results, and as none of the previously reported procedures showed promise for the determination of ferrous iron in solutions with concentrations smaller than 1 mg/l ferrous iron, it was decided to investigate one of the derivatives of 1,10-phenanthroline as the color-developing agent.

Bathophenanthroline Method

Laitinen and Nelson[16] obtained stable solutions of the ferrous-1,10-phenanthroline complex in the presence of ferric iron when methanol was used as the solvent. But this is of little value in determinations of ferrous iron in natural waters. It does, however, provide a clue as to the type of chromogenic agent desired.

Bathophenanthroline—4,7-diphenyl-1,10-phenanthroline—was selected for three reasons: (1) the reagent has increased sensitivity, approximately twice that of 1,10-phenanthroline; (2) the ferrous-bathophenanthroline complex is readily extracted by immiscible solvents—for example, isoamyl alcohol, nitrobenzene, and *n*-hexyl alcohol—which would tend to stabilize the developed colors; and (3) the extraction procedure renders the method less subject to interferences.

Bathophenanthroline was introduced as a colorimetric reagent for total iron by Smith, McCurdy, and Diehl.[17] They point out that this reagent should prove of value for the determination of small quantities of total iron in water supplies because it is more sensitive than the commonly used 1,10-phenanthroline. Knapp[18] recently published a modification of the original procedure of Smith and associates for the determination of 1 μg/l total iron in highly treated water.

The original procedure proposed by Smith and associates[17] for the determination of total iron has been modified in this investigation for the determination of ferrous iron. The modifications include the omission of the reducing agent and some changes in the volume of reagents and in the manner of purifying reagents.

One of the more important modifications concerns the amount of bathophenanthroline added to the samples. It was found that a larger excess of bathophenanthroline is needed to obtain stable colors for the determination of ferrous iron in the presence of ferric iron than was used[19] for the determination of total iron. The concentration of bathophenanthroline used in the suggested method was found suitable for total iron concentrations as large as 10 mg/l.

The procedure included in the suggested method consists of mixing the sample buffered at pH 4 with bathophenanthroline. The red ferrous-bathophenanthroline complex is extracted with *n*-hexyl alcohol, and the absorbance is determined spectrophotometrically. The *n*-hexyl alcohol, rather than isoamyl alcohol, was used as the immiscible solvent because much cleaner separations were obtained with it.

Results and Discussion

Typical absorbances for a series of ferrous iron and ferric iron solutions are shown in Table 1. These values were obtained with the procedure in the suggested method by using a spectrophotometer * at a wavelength of 533 mμ and a light path of 5.0 cm.

TABLE 1

Absorbance Values for Ferrous and Ferric Solutions

Concentration of Ferrous Iron per 50 ml of Solvent μg	Absorbance	Concentration of Ferric Iron per 50 ml of Solvent μg	Absorbance
2.65	0.112	10	0.021
5.30	0.224	20	0.032
10.6	0.454	30	0.040
15.9	0.694	40	0.048
21.2	0.935	50	0.057

It is of interest to compare the absorbance of ferrous iron with that of ferric iron. Examination of the values in Table 1 shows that ferric iron forms only slightly colored complexes with bathophenanthroline, and that unless large quantities of ferric iron are present its contribution to the absorbance can be neglected. A correction for large amounts of ferric iron can be made by establishing a calibration curve for ferric iron, as described in the procedure of the method, and obtaining the absorbance of aliquots of the sample run with and without hydroxylamine.

A series of eight runs was made, in which 11.70 μg ferrous iron was added to the separatory funnel. A mean iron concentration of 11.62 μg

* Model DU, made by Beckman Instruments, Fullerton, Calif.

was recovered with 10.8–12.1 μg ferrous iron. This procedure has been used successfully for the analysis of ferrous iron remaining as a function of time in an investigation of the kinetics of the oxidation of ferrous iron by oxygen.

One of the problems associated with the use of bathophenanthroline is its cost—$15 per gram in 1-gram lots,[19] or about 8 cents per analysis. Booth and Evett [20] suggested a procedure for the recovery of bathophenanthroline.

Summary

Many of the analytic procedures used for the determination of ferrous iron in the presence of ferric iron are not applicable to the determination of ferrous iron in natural waters because of a lack of sensitivity or because of the instability of developed color. The 1,10-phenanthroline method for the determination of ferrous iron, recommended in the ninth and tenth editions of *Standard Methods,* is likely to result in incorrect results unless it is applied in a very limited range and under special conditions.

A colorimetric procedure using bathophenanthroline as the color-developing reagent has been found to give reliable determinations of ferrous iron in the presence of ferric iron in quantities measured in micrograms.

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Suggested Procedure for the Specific Determination of Ferrous Iron

The method described here is a modification of a method recommended in two publications of the G. Frederick Smith Chemical Co., Columbus, Ohio.[19, 21]

1. General Discussion

None of the common anions—chloride, nitrate, acetate, or sulfate—interfere in the determination of iron with bathophenanthroline, nor do the alkali and alkaline-earth cations interfere. Large concentrations of perchlorate may cause precipitation of bathophenanthroline. Cobalt forms a light-yellow color with bathophenanthroline, but the color is not extracted from the aqueous solution. Copper forms a yellow complex with bathophenanthroline in neutral and alkaline solutions. At pH 4, the colored copper complex is not formed; thus copper does not interfere.

2. Reagents

2.1. *Bathophenanthroline,* 0.001*M* solution.* Prepare a 50 per cent ethyl alcohol solution of bathophenanthroline by dissolving 0.0332 g 4,7-diphenyl-1,10-phenanthroline ($C_{24}H_{16}N_2$, mol wt 332) in 50 ml of ethyl alcohol and diluting with 50 ml of iron-free water. Store this solution in a glass-stoppered reagent bottle.

2.2. *Hydroxylamine hydrochloride,* 10 per cent, iron-free solution. Reagent grade hydroxylamine often con-

* Solutions of bathophenanthroline and of the other reagents used in this analysis were made by G. Frederick Smith Chemical Co. The solutions are iron-free and ready for use.

tains appreciable amounts of iron. Dissolve 10 g hydroxylamine hydrochloride in 100 ml of distilled water in a 125-ml separatory funnel. Add 2 ml 0.001*M* bathophenanthroline and 10 ml *n*-hexyl alcohol, and extract. Repeat the extraction to insure complete removal of iron. Store the iron-free hydroxylamine hydrochloride solution in a glass-stoppered reagent bottle. This solution has a pH of 1.5–1.75. The small amount of *n*-hexyl alcohol left in the solution is not detrimental.

2.3. *Sodium acetate,* 10 per cent, iron-free solution. Dissolve 10 g sodium acetate in 100 ml of distilled water in a 125-ml separatory funnel. Add 2 ml 0.001*M* bathophenanthroline, and mix well. Add 10 ml *n*-hexyl alcohol, and shake vigorously. Allow the liquids to separate, and draw off the lower aqueous layer into a second separatory funnel. Repeat the separation to insure complete removal of iron. Store the solution in a glass-stoppered reagent bottle.

2.4. n-*Hexyl alcohol.* Reagent grade *n*-hexyl alcohol may be used without further purification. Technical-grade material must be distilled before use.

2.5. *Ethyl alcohol,* 95 per cent. Redistill commercial grade ethyl alcohol to remove iron.

2.6. *Standard iron solutions,* 10 μg/l and 1.0 μg/l Fe. Accurately weigh 0.0702 g ferrous ammonium sulfate hexahydrate (Mohr's salt), dissolve in distilled water that contains 2.5 ml concentrated H_2SO_4, and dilute to 1.0 liter. This solution contains 10 μg/ml Fe. Dilute 10 ml of this solution to 100 ml with distilled water that contains 2.5 ml concentrated H_2SO_4 per liter. This solution contains 1.0 μg/l Fe.

2.7. *Standard ferric iron solution,* 10 μg/ml Fe. Add excess aqueous chlorine to an aliquot of 10 μg/ml standard ferrous iron solution. Boil to remove unreacted aqueous chlorine. Dilute the cool solution to original aliquot volume.

3. Procedure

3.1. To obtain a sufficient quantity of the water sample, rinse the sample bottle several times with the water, fill the bottle completely full, and stopper in such a manner that no air bubble is left above the water. Add 2 ml of concentrated HCl per liter of sample. Avoid exposure of sample to atmospheric oxygen in the absence of strong acid.

3.2. Carry several solutions through the process together—for example, two samples of the unknown to be analyzed, three or four standards, and a blank. Once a calibration curve has been established, further standards need not be run except as an occasional check.

3.3. The determination of ferrous iron in natural waters requires boiling of the sample in the presence of a strong acid to free the iron from organic matter or from mixed ferrous-ferric oxides. This can usually be accomplished by adding 1 ml of concentrated iron-free HCl per 25 ml of sample and boiling for approximately 5 min. A weak base such as sodium acetate should be used to neutralize the excess acid.

3.4. For the standards, use various amounts of the second standard iron solution (1 μg/ml)—for example: 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 . . . and 20 ml, corresponding to 2.00, 4.00 . . . and 20.0 μg of iron. The volumes of reagents are based on a 5-cm light

path. Appropriate modifications can be made for other cell lengths by varying the concentrations of the iron in the reference solutions.

3.5. Pipet the sample (water being tested or standard iron solution) into a 125-ml separatory funnel. The pH should be less than 1.5. If the sample taken was less than 10 ml, add sufficient distilled water to bring the volume to 10 ml. Larger volumes may be used. Use 10 ml of distilled water for the blank.

3.6. To each sample, add 4 ml of 10 per cent sodium acetate solution. The solution at this point should be pH 4. If the original sample of the water was acidified with HCl when taken, add an additional 4 ml of sodium acetate solution. Add 2 ml of hydroxylamine hydrochloride solution to standard iron solutions. (Omit the hydroxylamine in waters being tested for ferrous iron and in the ferric iron standards.)

3.7. Add 15 ml 0.001*M* bathophenanthroline, and mix. Add 10.0 ml *n*-hexyl alcohol, stopper the funnel, and shake the mixture thoroughly. Allow the liquids to separate for at least 5 min after shaking.

3.8. After the liquids have cleanly separated into two layers, draw off and discard the lower aqueous layer. Shake away any of the aqueous layer remaining in the stem of the separatory funnel. Drain the *n*-hexyl alcohol layer into a 50-ml volumetric flask—a smaller-volume flask may be used to increase the sensitivity—and wash out the separatory funnel with 2–3 ml of ethyl alcohol added from a pipet. This should be done in such a manner that the upper stopper of the funnel and the walls of the funnel are uniformly washed at least twice by a film of alcohol as it drains from the top to the bottom. Transfer this wash alcohol to the volumetric flask. Dilute the solution in the flask to the mark with ethyl alcohol, and mix by shaking. At this point the solution in the volumetric flask should be clear, with no turbidity.

3.9. Determine the absorbance of the solution within 10 min. Avoid exposure of solution to strong sunlight, making the measurement at a wavelength of 533 mμ. The iron-bathophenanthroline color conforms to Beer's law, so that a plot of absorbance (negative logarithm of transmittance) against concentration of iron yields a straight line with the slope equal to the absorptivity.

If the blank turns out to be large, owing to iron in the distilled water or reagents, it may be necessary to repeat the work using water or reagents that have been freed of iron in the same manner as the sodium acetate solution. The procedure described can be used for the determination of total iron by addition of 2 ml of hydroxylamine hydrochloride to the iron samples prior to the addition of bathophenanthroline.

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