3500-Fe IRON*#(1)

3500-Fe A. Introduction

1. Occurrence and Significance

Iron (Fe) is the first element in Group VIII of the periodic table; it has an atomic number of 26, an atomic weight of 55.85, and common valences of 2 and 3 (and occasionally valences of 1, 4, and 6). The average abundance of Fe in the earth's crust is 6.22%; in soils Fe ranges from 0.5 to 4.3%; in streams it averages about 0.7 mg/L; and in groundwater it is 0.1 to 10 mg/L. Iron occurs in the minerals hematite, magnetite, taconite, and pyrite. It is widely used in steel and in other alloys.

The solubility of ferrous ion (Fe^{2+}) is controlled by the carbonate concentration. Because groundwater is often anoxic, any soluble iron in groundwater is usually in the ferrous state. On exposure to air or addition of oxidants, ferrous iron is oxidized to the ferric state (Fe^{3+}) and may hydrolyze to form red, insoluble hydrated ferric oxide. In the absence of complex-forming ions, ferric iron is not significantly soluble unless the pH is very low.

Elevated iron levels in water can cause stains in plumbing, laundry, and cooking utensils, and can impart objectionable tastes and colors to foods. The United Nations Food and Agriculture Organization recommended level for irrigation waters is 5 mg/L. The U.S. EPA secondary drinking water standard MCL is 0.3 mg/L.

2. Selection of Method

Sensitivity and detection limits for the atomic absorption spectrometric methods (Section 3111B and Section 3111C), the inductively coupled plasma method (Section 3120), and the phenanthroline colorimetric procedure described here (B) are similar and generally adequate for analysis of natural or treated waters. Lower detection limits can be achieved with electrothermal atomic absorption spectrometry (Section 3113B) when an appropriate matrix modifier is used. The complexing reagents used in the colorimetric procedures are specific for ferrous iron but the atomic absorption procedures are not. However, because of the instability of ferrous iron, which is changed easily to the ferric form in solutions in contact with air, determination of ferrous iron requires special precautions and may need to be done in the field at the time of sample collection.

The procedure for determining ferrous iron using 1,10-phenanthroline (Section 3500-Fe.B.4*c*) has a somewhat limited applicability; avoid long storage time or exposure of samples to light. A rigorous quantitative distinction between ferrous and ferric iron can be obtained with a special procedure using bathophenanthroline. Spectrophotometric methods using bathophenanthroline[1-6] and other organic complexing reagents such as ferrozine[7] or TPTZ[8] are capable of determining iron concentrations as low as 1 µg/L. A chemiluminescence procedure[9] is stated to have a

detection limit of 5 ng/L. Additional procedures are described elsewhere.[10-13]

3. Sampling and Storage

Plan in advance the methods of collecting, storing, and pretreating samples. Clean sample container with acid and rinse with reagent water. Equipment for membrane filtration of samples in the field may be required to determine iron in solution (dissolved iron). Dissolved iron, considered to be that passing through a 0.45-µm membrane filter, may include colloidal iron. The value of the determination depends greatly on the care taken to obtain a representative sample. Iron in well or tap water samples may vary in concentration and form with duration and degree of flushing before and during sampling. When taking a sample portion for determining iron in suspension, shake the sample bottle often and vigorously to obtain a uniform suspension of precipitated iron. Use particular care when colloidal iron adheres to the sample bottle. This problem can be acute with plastic bottles.

For a precise determination of total iron, use a separate container for sample collection. Treat with acid at the time of collection to place the iron in solution and prevent adsorption or deposition on the walls of the sample container. Take account of the added acid in measuring portions for analysis. The addition of acid to the sample may eliminate the need for adding acid before digestion (Section 3500-Fe.B.4*a*).

4. References

1. LEE, G.F. & W. STUMM. 1960. Determination of ferrous iron in the presence of ferric iron using bathophenanthroline. *J. Amer. Water Works Assoc.* 52:1567.
2. GHOSH, M.M., J.T. O'CONNOR & R.S. ENGELBRECHT. 1967. Bathophenanthroline method for the determination of ferrous iron. *J. Amer. Water Works Assoc.* 59:897.
3. BLAIR, D. & H. DIEHL. 1961. Bathophenanthroline-disulfonic acid and bathocuproine-disulfonic acid, water soluble reagents for iron and copper. *Talanta* 7:163.
4. SHAPIRO, J. 1966. On the measurement of ferrous iron in natural waters. *Limnol. Oceanogr.* 11:293.
5. MCMAHON, J.W. 1967. The influence of light and acid on the measurement of ferrous iron in lake water. *Limnol. Oceanogr.* 12:437.
6. MCMAHON, J.W. 1969. An acid-free bathophenanthroline method for measuring dissolved ferrous iron in lake water. *Water Res.* 3:743.
7. GIBBS, C. 1976. Characterization and application of ferrozine iron reagent as a ferrous iron indicator. *Anal. Chem.* 48:1197.
8. DOUGAN, W.K. & A.L. WILSON. 1973. Absorbtiometric determination of iron with TPTZ. *Water Treat. Exam.* 22:110.
9. SEITZ, W.R. & D.M. HERCULES. 1972. Determination of trace amounts of iron (II) using chemiluminescence analysis. *Anal. Chem.* 44:2143.

10. MOSS, M.L. & M.G. MELLON. 1942. Colorimetric determination of iron with 2,2′-bipyridine and with 2,2′,2′′-tripyridine. *Ind. Eng. Chem.,* Anal. Ed. 14:862.
11. WELCHER, F.J. 1947. Organic Analytical Reagents. D. Van Nostrand Co., Princeton, N.J., Vol. 3, pp. 100–104.
12. MORRIS, R.L. 1952. Determination of iron in water in the presence of heavy metals. *Anal. Chem.* 24:1376.
13. DOIG, M.T., III & D.F. MARTIN. 1971. Effect of humic acids on iron analyses in natural water. *Water Res.* 5:689.

3500-Fe B. Phenanthroline Method

1. General Discussion

a. Principle: Iron is brought into solution, reduced to the ferrous state by boiling with acid and hydroxylamine, and treated with 1,10-phenanthroline at pH 3.2 to 3.3. Three molecules of phenanthroline chelate each atom of ferrous iron to form an orange-red complex. The colored solution obeys Beer's law; its intensity is independent of pH from 3 to 9. A pH between 2.9 and 3.5 insures rapid color development in the presence of an excess of phenanthroline. Color standards are stable for at least 6 months.

b. Interference: Among the interfering substances are strong oxidizing agents, cyanide, nitrite, and phosphates (polyphosphates more so than orthophosphate), chromium, zinc in concentrations exceeding 10 times that of iron, cobalt and copper in excess of 5 mg/L, and nickel in excess of 2 mg/L. Bismuth, cadmium, mercury, molybdate, and silver precipitate phenanthroline. The initial boiling with acid converts polyphosphates to orthophosphate and removes cyanide and nitrite that otherwise would interfere. Adding excess hydroxylamine eliminates errors caused by excessive concentrations of strong oxidizing reagents. In the presence of interfering metal ions, use a larger excess of phenanthroline to replace that complexed by the interfering metals. Where excessive concentrations of interfering metal ions are present, the extraction method may be used.

If noticeable amounts of color or organic matter are present, it may be necessary to evaporate the sample, gently ash the residue, and redissolve in acid. The ashing may be carried out in silica, porcelain, or platinum crucibles that have been boiled for several hours in 6*N* HCl. The presence of excessive amounts of organic matter may necessitate digestion before use of the extraction procedure.

c. Minimum detectable concentration: Dissolved or total concentrations of iron as low as 10 µg/L can be determined with a spectrophotometer using cells with a 5 cm or longer light path.

Carry a blank through the entire procedure to allow for correction.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 510 nm, providing a light path of 1 cm or longer.

2) *Filter photometer,* providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 510 nm.

3) *Nessler tubes,* matched, 100-mL, tall form.

b. Acid-washed glassware: Wash all glassware with conc hydrochloric acid (HCl) and rinse with reagent water before use to remove deposits of iron oxide.

c. Separatory funnels: 125-mL, Squibb form, with ground-glass or TFE stopcocks and stoppers.

3. Reagents

Use reagents low in iron. Use reagent water (see Section 1080 and Section 3111B.3*c*) in preparing standards and reagent solutions and in procedure. Store reagents in glass-stoppered bottles. The HCl and ammonium acetate solutions are stable indefinitely if tightly stoppered. The hydroxylamine, phenanthroline, and stock iron solutions are stable for several months. The standard iron solutions are not stable; prepare daily as needed by diluting the stock solution. Visual standards in nessler tubes are stable for several months if sealed and protected from light.

a. Hydrochloric acid, HCl, conc, containing less than 0.5 ppm iron.

b. Hydroxylamine solution: Dissolve 10 g $NH_2OH{\cdot}HCl$ in 100 mL water.

c. Ammonium acetate buffer solution: Dissolve 250 g $NH_4C_2H_3O_2$ in 150 mL water. Add 700 mL conc (glacial) acetic acid. Because even a good grade of $NH_4C_2H_3O_2$ contains a significant amount of iron, prepare new reference standards with each buffer preparation.

d. Sodium acetate solution: Dissolve 200 g $NaC_2H_3O_2{\cdot}3H_2O$ in 800 mL water.

e. Phenanthroline solution: Dissolve 100 mg 1,10-phenanthroline monohydrate, $C_{12}H_8N_2{\cdot}H_2O$, in 100 mL water by stirring and heating to 80°C. Do not boil. Discard the solution if it darkens. Heating is unnecessary if 2 drops conc HCl are added to the water. (NOTE: One milliliter of this reagent is sufficient for no more than 100 µg Fe.)

f. Potassium permanganate, 0.1*M:* Dissolve 0.316 $KMnO_4$ in reagent water and dilute to 100 mL.

g. Stock iron solution: Use metal (1) or salt (2) for preparing the stock solution.

1) Use electrolytic iron wire, or ‘‘iron wire for standardizing,’’ to prepare the solution. If necessary, clean wire with fine sandpaper to remove any oxide coating and to produce a bright surface. Weigh 200.0 mg wire and place in a 1000-mL volumetric flask. Dissolve in 20 mL 6*N* sulfuric acid (H_2SO_4) and dilute to mark with water; 1.00 mL = 200 µg Fe.

2) If ferrous ammonium sulfate is preferred, slowly add 20 mL conc H_2SO_4 to 50 mL water

and dissolve 1.404 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$. Add 0.1*M* potassium permanganate ($KMnO_4$) dropwise until a faint pink color persists. Dilute to 1000 mL with water and mix; 1.00 mL = 200 µg Fe.

h. Standard iron solutions: Prepare daily for use.

1) Pipet 50.00 mL stock solution into a 1000-mL volumetric flask and dilute to mark with water; 1.00 mL = 10.0 µg Fe.

2) Pipet 5.00 mL stock solution into a 1000-mL volumetric flask and dilute to mark with water; 1.00 mL = 1.00 µg Fe.

i. Diisopropyl or isopropyl ether. CAUTION: *Ethers may form explosive peroxides; test before using.*

4. Procedure

a. Total iron: Mix sample thoroughly and measure 50.0 mL into a 125-mL erlenmeyer flask. If this sample volume contains more than 200 µg iron use a smaller accurately measured portion and dilute to 50.0 mL. Add 2 mL conc HCl and 1 mL $NH_2OH \cdot HCl$ solution. Add a few glass beads and heat to boiling. To insure dissolution of all the iron, continue boiling until volume is reduced to 15 to 20 mL. (If the sample is ashed, take up residue in 2 mL conc HCl and 5 mL water.) Cool to room temperature and transfer to a 50- or 100-mL volumetric flask or nessler tube. Add 10 mL $NH_4C_2H_3O_2$ buffer solution and 4 mL phenanthroline solution, and dilute to mark with water. Mix thoroughly and allow a minimum of 10 min for maximum color development.

b. Dissolved iron: Immediately after collection filter sample through a 0.45-µm membrane filter into a vacuum flask containing 1 mL conc HCl/100 mL sample. Analyze filtrate for total dissolved iron (¶ 4*a*) and/or dissolved ferrous iron (¶ 4*c*). (This procedure also can be used in the laboratory if it is understood that normal sample exposure to air during shipment may result in precipitation of iron.)

Calculate suspended iron by subtracting dissolved from total iron.

c. Ferrous iron: Determine ferrous iron at sampling site because of the possibility of change in the ferrous-ferric ratio with time in acid solutions. To determine ferrous iron only, acidify a separate sample with 2 mL conc HCl/100 mL sample at time of collection. Fill bottle directly from sampling source and stopper. Immediately withdraw a 50-mL portion of acidified sample and add 20 mL phenanthroline solution and 10 mL $NH_4C_2H_3O_2$ solution with vigorous stirring. Dilute to 100 mL and measure color intensity within 5 to 10 min. Do not expose to sunlight. (Color development is rapid in the presence of excess phenanthroline. The phenanthroline volume given is suitable for less than 50 µg total iron; if larger amounts are present, use a correspondingly larger volume of phenanthroline or a more concentrated reagent.)

Calculate ferric iron by subtracting ferrous from total iron.

d. Color measurement: Prepare a series of standards by accurately pipetting calculated volumes of standard iron solutions [use solution described in ¶ 3*h*2) to measure 1- to 10-µg portions] into 125-mL erlenmeyer flasks, diluting to 50 mL by adding measured volumes of water, and carrying out the steps in ¶ 4*a* beginning with transfer to a 100-mL volumetric flask or nessler tube.

For visual comparison, prepare a set of at least 10 standards, ranging from 1 to 100 µg Fe in the final 100-mL volume. Compare colors in 100-mL tall-form nessler tubes.

For photometric measurement, use Table 3500-Fe:I as a rough guide for selecting proper light path at 510 nm. Read standards against water set at zero absorbance and plot a calibration curve, including a blank (see ¶ 3*c* and General Introduction).

If samples are colored or turbid, carry a second set of samples through all steps of the procedure without adding phenanthroline. Instead of water, use the prepared blanks to set photometer to zero absorbance and read each sample developed with phenanthroline against the corresponding blank without phenanthroline. Translate observed photometer readings into iron values by means of the calibration curve. This procedure does *not* compensate for interfering ions.

e. Samples containing organic interferences: Digest samples containing substantial amounts of organic substances according to the directions given in Section 3030G or Section 3030H.

1) If a digested sample has been prepared according to the directions given in Section 3030G or Section 3030H, pipet 10.0 mL or other suitable portion containing 20 to 500 µg Fe into a 125-mL separatory funnel. If the volume taken is less than 10 mL, add water to make up to 10 mL. To the separatory funnel add 15 mL conc HCl for a 10-mL aqueous volume; or, if the portion taken was greater than 10.0 mL, add 1.5 mL conc HCl/mL sample. Mix, cool, and proceed with ¶ 4*e*3) below.

2) To prepare a sample solely for determining iron, measure a suitable volume containing 20 to 500 µg Fe and carry it through the digestion procedure described in either Section 3030G or Section 3030H. However, use only 5 mL H_2SO_4 or $HClO_4$ and omit H_2O_2. When digestion is complete, cool, dilute with 10 mL water, heat almost to boiling to dissolve slowly soluble salts, and, if the sample is still cloudy, filter through a glass-fiber, sintered-glass, or porcelain filter, washing with 2 to 3 mL water. Quantitatively transfer filtrate or clear solution to a 25-mL volumetric flask and make up to 25 mL with water. Empty flask into a 125-mL separatory funnel, rinse flask with 5 mL conc HCl and add to the funnel. Add 25 mL conc HCl measured with the same flask. Mix and cool to room temperature.

3) Extract the iron from the HCl solution in the separatory funnel by shaking for 30 s with 25 mL isopropyl ether (CAUTION). Draw off lower acid layer into a second separatory funnel. Extract acid solution again with 25 mL isopropyl ether, drain acid layer into a suitable clean vessel, and add ether layer to the ether in the first funnel. Pour acid layer back into second separatory funnel and re-extract with 25 mL isopropyl ether. Withdraw and discard acid layer and add ether layer to first funnel. Persistence of a yellow color in the HCl solution after three extractions does not signify incomplete separation of iron because copper, which is not extracted,

gives a similar yellow color.

Shake combined ether extracts with 25 mL water to return iron to aqueous phase and transfer lower aqueous layer to a 100-mL volumetric flask. Repeat extraction with a second 25-mL portion of water, adding this to the first aqueous extract. Discard ether layer.

4) Add 1 mL $NH_2OH \cdot HCl$ solution, 10 mL phenanthroline solution, and 10 mL $NaC_2H_3O_2$ solution. Dilute to 100 mL with water, mix thoroughly, and let stand for a minimum of 10 min. Measure absorbance at 510 nm using a 5-cm absorption cell for amounts of iron less than 100 µg or 1-cm cell for quantities from 100 to 500 µg. As reference, use either water or a sample blank prepared by carrying the specified quantities of acids through the entire analytical procedure. If water is used as reference, correct sample absorbance by subtracting absorbance of a sample blank.

Determine micrograms of iron in the sample from the absorbance (corrected, if necessary) by reference to the calibration curve prepared by using a suitable range of iron standards containing the same amounts of phenanthroline, hydroxylamine, and sodium acetate as the sample.

5. Calculation

When the sample has been treated according to ¶ 4*a*, ¶ 4*b*, ¶ 4*c*, or ¶ 4*e*2):

$$\text{mg Fe/L} = \frac{\text{µg Fe (in 100 mL final volume)}}{\text{mL sample}}$$

When the sample has been treated according to ¶ 4*e*1):

$$\text{mg Fe/L} = \frac{\text{µg Fe (in 100 mL final volume)}}{\text{mL sample}} \times \frac{100}{\text{mL portion}}$$

Report details of sample collection, storage, and pretreatment if they are pertinent to interpretation of results.

6. Precision and Bias

Precision and bias depend on the method of sample collection and storage, the method of color measurement, the iron concentration, and the presence of interfering color, turbidity, and foreign ions. In general, optimum reliability of visual comparison in nessler tubes is not better than 5% and often only 10%, whereas, under optimum conditions, photometric measurement may be reliable to 3% or 3 µg, whichever is greater. The sensitivity limit for visual observation in nessler tubes is approximately 1 µg Fe. Sample variability and instability may affect precision and bias of this determination more than will the errors of analysis. Serious divergences have been found in reports of different laboratories because of variations in methods of collecting and treating

samples.

A synthetic sample containing 300 µg Fe/L, 500 µg Al/L, 50 µg Cd/L, 110 µg Cr/L, 470 µg Cu/L, 70 µg Pb/L, 120 µg Mn/L, 150 µg Ag/L, and 650 µg Zn/L in distilled water was analyzed in 44 laboratories by the phenanthroline method, with a relative standard deviation of 25.5% and a relative error of 13.3%.

7. Bibliography

CHRONHEIM, G. & W. WINK. 1942. Determination of divalent iron (by o-nitrosophenol). *Ind. Eng. Chem.,* Anal. Ed. 14:447.

MEHLIG, R.P. & R.H. HULETT. 1942. Spectrophotometric determination of iron with o-phenanthroline and with nitro-o-phenanthroline. *Ind. Eng. Chem.,* Anal. Ed. 14:869.

CALDWELL, D.H. & R.B. ADAMS. 1946. Colorimetric determination of iron in water with o-phenanthroline. *J. Amer. Water Works Assoc.* 38: 727.

WELCHER, F.J. 1947. Organic Analytical Reagents. D. Van Nostrand Co., Princeton, N.J., Vol. 3, pp. 85–93.

KOLTHOFF, I.M., T.S. LEE & D.L. LEUSSING. 1948. Equilibrium and kinetic studies on the formation and dissociation of ferroin and ferrin. *Anal. Chem.* 20:985.

RYAN, J.A. & G.H. BOTHAM. 1949. Iron in aluminum alloys: Colorimetric determination using 1,10-phenanthroline. *Anal. Chem.* 21:1521.

REITZ, L.K., A.S. O'BRIEN & T.L. DAVIS. 1950. Evaluation of three iron methods using a factorial experiment. *Anal. Chem.* 22:1470.

SANDELL, E.B. 1959. Chapter 22 *in* Colorimetric Determination of Traces of Metals, 3rd ed. Interscience Publishers, New York, N.Y.

SKOUGSTAD, M.W., M.J. FISHMAN, L.C. FRIEDMAN, D.E. ERDMANN & S.S. DUNCAN. 1979. Methods for Determination of Inorganic Substances in Water and Fluvial Sediment. Chapter A1 *in* Book 5, Techniques of Water Resources Investigations of the United States Geological Survey. U.S. Geological Surv., Washington, D.C.

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Endnotes

1 (Popup - Footnote)

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