# 1,10-PHENANTHROLINE METHOD FOR Fe(II) AND Fe(III)

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## A. REAGENTS

1. 10 % (w/w) 1,10-phenanthroline monohydrate in 95 % ethanol (denoted hereafter as phen) (Sigma Chemical, melting point 90-100 $^{o}$C, 6-9 % $H_2O$; other vendors' products have proven unsatisfactory)
2. 3.6 N sulfuric acid ($H_2SO_4$) in 250-500 mL dispensing bottle
3. 48 % hydrofluoric acid (HF), kept in hood in plastic dispensing bottle (HF dissolves glass)
4. 5 % (w/w) boric acid ($H_3BO_4$) in water in a 1-2 L dispensing bottle
5. 1 % (w/w) sodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$) in water
6. 10 % hydroxiylamine hydrochloride solution in water
7. Ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$) salt for standards
8. High-purity water (preferably with a resistance of 18 megohm or more) for all dilutions and solutions

## B. EQUIPMENT

1. Brinkmann Dosimat dilutor
2. Varian Cary 5 UV-VIS spectrophotometer.
3. High-intensity mercury vapor lamp.
4. Hot plate.
5. Pan for boiling water on hot plate and rust-proof holding rack for digestion tubes.
6. Digestion tubes: either 100-mL polypropylene centrifuge tubes or 50-mL polycarbonate Oak Ridge-type tubes
7. 125-mL Pyrex Erlenmeyer flasks
8. Balance with at least 0.0001 g resolution

## C. PROCEDURE

1. Turn on hot plate in hood; add water to about 3-4 inches depth.
2. Use at least three digestion tubes for standards, including one blank and two containing appropriate amounts of $Fe(NH_4)_2(SO_4)_2.6H_2O$ (approximately 7 mg/ppm Fe in final dilution if using 100 mL digestion tubes; or 3.08 mg/ppm Fe desired in final dilution if using 40 mL tubes); and one tube for each sample. Place tubes in rack.
3. Record the weight of each empty tube.
4. Add standard or sample quantitatively to each tube, and record the combined weight of tube + sample (or standard).
5. Check all reagents for proper amounts. Be sure that all volume adjustments are proper. Pump each at least once into waste beaker.
6. Turn off room lights. Turn on red lamps at both counters and at balance.
7. To each tube add 12 mL $H_2SO_4$ (12 mL corresponds to two strokes with the dispening pipet in the dark room), being sure to wash down any sample that may be clinging to the tube wall; followed immediately by 2 mL 10% phen. A precision of ± 1-5 % in these transfers is sufficient.

8. Place rack with tubes in hood and add 1 mL 48 % HF.
9. Place rack with tubes in boiling water bath for 30 min.
10. While boiling, set up on a moveable cart two-erlenmeyer flasks for each digestion tube, and cut parafilm for flasks and tubes.
11. Remove samples from boiling water bath and allow to cool for 15 min.
12. While cooling, prepare the UV-visible spectrophotometer.
13. Add 10 mL $H_3BO_4$ to each tube.
14. Dilute all tubes to approximately equal volumes with water, to within about ½ inch of the rim.
15. Weigh each tube on the balance and record the weight of the tube + sample + solution (record the maximum weight because there is weight loss due to evaporation). Be sure to correct for the weight of the tube holder on the balance pan.
16. Cover each tube with parafilm and invert 3 times, being sure the solution is well mixed.
17. Using the Dosimat automatic dilutor, transfer one aliquot of each sample to each of two flasks and dilute to final volume (21.88648 g).
    a. Fill the Dosimat bottle with 1 % Na-citrate solution.
    b. Clean the pipet tip with water and wipe dry with a Chimwipe.
    c. Fill a 50-mL beaker with water for rinsing and cleaning the pipet tip between samples.
    d. Insert the pipet tip into the water, press the pipette button to pipet the 2 mL of water. Move the pipet tip over a discard beaker and press the button once more. The entire solution (about 22 mL) should then be expelled into the beaker.
    e. Rinse and wipe the tip.
    f. Be sure **Dil 1 2.0000 mL** is displayed, then place pipet tip into the first digestion tube. Press the pipette button. When **Dil 2** appears on the display, move the pipet tip to the first flask and press the pipette button again. Cover the flask with parafilm. Repeat this process, transferring another aliquot from the first digestion tube to the second flask. Rinse pipet tip and dry with Chimwipe.
    g. Repeat step f for each digestion tube.
    h. To prepare the total iron analysis it is necessary to change the dilution volume to 19 mL. On the keyboard press volume 1 + 9 and enter. The final volume must be 21 mL. With the Eppendorf pipette transfer 1 mL of 10 % hydroxylamine hydrochloride in the flasks
    i. Cover the glass flasks with parafilm and leave them for 24 h (or overnight) in the darkroom to complete reduction.
18. Cover sample tubes and set aside as a precaution in the event a flask is ruined.
19. Turn off lights in room where the UV-visible spectrophotometer is located. Turn on red lamp over the instrument. Transfer samples to this room.
20. Swirl each flask 3 times, sip a portion into the spectrophotometer flow cell, and record the absorbance as per **UV-Visible Data Collection** procedures.
21. Measure the absorbance at 510 nm for the total Fe flasks.
22. Calculate results as per **UV-Visible Data Collection** procedures.