Spectrophotometric Determination of Iron Species in Natural Waters

CHEM 467 – Current Microanalytical Methods

Objectives:

(1) Learn the principles of colorimetric and spectrophotometric methods of analysis.
(2) Analyze the absorption spectra of two different iron complexes
(3) Obtaining and interpreting a Beer's Law Plot from prepared standard solutions
(4) Iron determination in natural water samples using Beer's Law Plot

Instrumentation:

Varian 300 Bio UV-Visible Spectrophotometer

Background and Principles:

Iron is a vital element for many animals including humans where it has a significant role in the respiratory process. Here, iron containing proteins transport oxygen and play an important role in the body's energy-generating process.[1] Iron is also an essential micronutrient for organisms and in certain high-nutrient, low-chlorophyll portions of the world's oceans.[2] Both iron(II) and iron(III) forms are found to be dissolved in water. Large concentrations of iron, however, can cause deleterious effects on aquatic life. It is thus vitally important to be able to measure such levels quantitatively and reproducibly. A simple, but sensitive method for the colorimetric determination of iron involves chelating iron with three equivalents of 1,10-phenanthroline (phen) in a buffered solution with low pH:

$$Fe^{2+} + 3\ phen \rightarrow [Fe(phen)_3]^{2+}$$

The orange-red complex has a λ_{max} = 510 nm. In this process, hydroxylamine hydrochloride is added to reduce iron(III) to iron(II)—the effective complexing species.[1] Excess phen is added at pH between 3.5 – 4.5, a range that aids in color development.

The Lambert-Beer Law[3] is fundamental to colorimetric and spectrophotometric methods of detection:

$$A = \varepsilon bc$$

where A = absorbance, ε = molar absorptivity, b = cell path length (cm), and c = concentration (mol L^{-1}). Detailed descriptions of the spectrophotometric process will be covered in lecture.

Procedures:

A: Absorption spectrum

1. Mix together 5 mL each of 0.01 M KSCN (prepare fresh daily) and 0.01 M $Fe(NO_3)_2$.
2. Repeat step 1 using 0.01 M $Fe(NO_2)_2$ (with addition of acid – 1 mL per 100 mL) in place of 0.01 M ferrous ion.
3. Using the spectrophotometer, measure and record the absorption spectra scanning from 760-400 nm. Note: dilution may be necessary to bring absorbance within range of the instrument. Use water as a reference in your analysis.

4. Repeat steps 1 and 3 using Fe(II) nitrate and 5 mL of 0.3% 1,10-phenanthroline solutions instead of thiocyanate. What are the differences if any?

B: Preparation of Standards

1. Stock solution – weigh 351 mg of ferrous ammonium sulfate hexahydrate, $FeSO_4 \bullet (NH_4)_2SO_4 \bullet 6H_2O$ and quantitatively transfer to a 500 mL volumetric flask. Add 50 mL of distilled H_2O (DI) and 1 mL of concentrated sulfuric acid. Dilute to the mark with DI. This is a 100 ppm solution.
2. Prepare standard iron(II) solutions having concentrations of 0, 0.5, 1.0, 2.0 and 5.0 ppm from the stock solution in step 1.

C: Beer's Law Plot

Transfer a 5 mL aliquot of the 0 ppm standard to a 125 mL Erlenmeyer flask and test the pH with test paper. If greater than 4.5, add enough 0.1 M H_2SO_4 (prepare) dropwise to adjust pH to 3.5. Add the same number of drops of sodium citrate (259 g L^{-1}, prepare) to bring the pH back to 4.5. Pipet 1 mL of 10% hydroxylamine hydrochloride and 3 mL of phen to the sample, mix and allow color development for 5 min. Use the same number of drops of H_2SO_4 and sodium citrate to the remaining standards plus the 1 mL of 10% hydroxylamine hydrochloride and 3 mL phen. Mix well and let develop for 5 min. Using water as a reference, measure the absorbance of each standard (3 repetitions for each standard) and record. Prepare a Beer's Law Plot.

D: Analysis of Samples

Using the same procedure as the standards, measure the absorbance of your natural water samples (3 repetitions) and record.

WASTE: ***Do not put KSCN down the drain***. Please use container provided by the instructors. All others can go down the drain with a water flush.

Data Analysis and Interpretation:

1. From the two absorption spectra generated, discuss the effect of the ligand on the wavelength and maximum absorbance of the peaks obtained.
2. Prepare a Beer's Law Plot for the standard solutions prepared. Carry out a least-squares analysis and determine the slop, the *y*-intercept and the correlation coefficient for the best straight line.
3. Use the Beer's Plot generated to determine the concentration of iron in each water sample analyzed. Distinguish between the two ways of determining concentration from the plot.
4. Report the mean, standard deviation and relative standard deviation of your measurements.

Literature Cited:

1. D. N. Boehnke and R.D. Delumyea, *Laboratory Experiments in Environmental Chemistry*, Prentice Hall, New Jersey, 2000.

2. A.R. Bowie, E.P. Achterberg, R.F.C. Mantoura and P.J. Worsfold, (1998) Determination of sub-nanomolar levels of iron is seawater using flow injection with chemiluminescence detection, *Analytica Chimica Acta*, 361, 189-200.
3. D.A. Skoog, D.M. West, F.J. Holler and S.R. Crouch, Fundamentals of Analytical Chemistry, 8th Edition, Sanders, New York, 2004.