Experiment 9

Spectrophotometry, Colorimetry, and Absorption Spectra: Determining Iron and Manganese in Natural Waters and Sediments

Objectives—The objectives of this experiment are:

1. To illustrate the general principles of absorption spectrophotometry by demonstrating Beer's law
2. To measure the concentration of iron in water and manganese in soil or sediment

Introduction—Colorimetric and spectrophotometric methods are perhaps the most frequently used and important methods of quantitative analysis. These methods are based on the absorption of light by a sample. The amount of radiant energy absorbed is proportional to the concentration of the absorbing material, and by measuring the absorption of radiant energy, it is possible to determine quantitatively the amount of substance present.

Colorimetric and spectrophotometric methods of analysis have been worked out for most of the elements and for many types of organic compounds. Methods based on the absorption of light are well suited to the determination of sample constituents from trace levels up to amounts of 1–2% but are not as frequently used for the analysis of larger (macro) quantities of substances.

Theory—The fundamental law on which colorimetric and spectrophotometric methods are based is the Bouguer-Beer or Lambert-Beer law, usually referred to simply as Beer's law. In mathematical form this law is

$$A = abc \qquad \textbf{(9-1)}$$

where A is the absorbance, a is the absorptivity, b is the internal cell length, and c is the concentration of the solution. When the concentration is expressed in mol/L, Beer's law is written

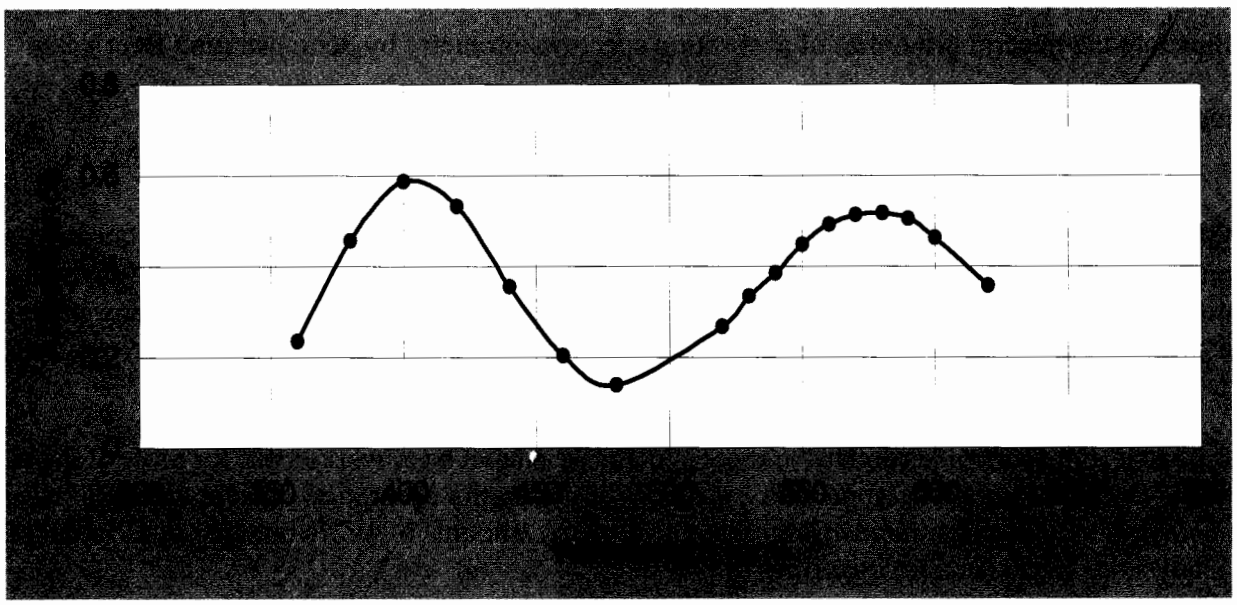
$$A = \varepsilon bC \qquad \textbf{(9-2)}$$

where ε is called the molar absorptivity, or the extinction coefficient, and C is the molarity. Typically, b is measured in cm, and therefore ε has units of $M^{-1}cm^{-1}$.

The colorimeter, or spectrophotometer, is an important analytical instrument that makes possible a quantitative measurement of the light that passes through a solution. The first step in an analysis is the

determination of the optimum wavelength to use for the analysis. The analyte must appreciably absorb light at the wavelength chosen. In a colorimeter exact wavelengths are not used, but rather small bands of wavelengths, and the wavelength chosen for analysis must be such that the absorbance does not change rapidly with the wavelength. If all the wavelengths in this narrow band are absorbed to nearly the same extent, the result is the same as if we isolated a single wavelength to use. Therefore, for an analysis we choose a flat portion of the absorption spectrum (a plot of absorbance versus wavelength). An absorption spectrum is shown in Figure 9-1.

Figure 9-1 Absorption Spectrum of Chromium (III) Nitrate

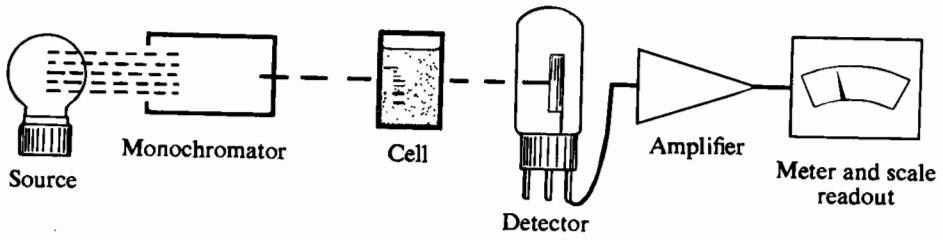


Instruments that measure the absorption of radiant energy, spectrophotometers, have five essential components, as shown in Figure 9-2. For instruments that are used in the visible region of the spectrum, a tungsten filament bulb is used as the source. The **wavelength** of light that enters the system is limited by means of a filter or a monochromator. The **amount** of light that enters the system is controlled with a variable slit or other means. The light then passes through the sample solution held in a glass cell called a cuvette (quartz must be used in the ultraviolet region). Finally, the transmitted light strikes a phototube, or other transducer (such as a photodiode), that converts it into an electric current. The current produced is a function of the radiant power of the light striking the transducer. The current is amplified and is then measured by a meter or a digital readout.

The advantage of a colorimeter is its relatively low cost and simplicity of operation. However, most colorimeters are not able to automatically change wavelength. The output from the source is not constant for all wavelengths, and this necessitates an adjustment in slit width or the sensitivity whenever the wavelength is changed. Also, colorimeters are single-beam instruments and therefore cannot automatically correct for the intensity changes in the light source and variations in detector sensitivity when the wavelength is changed.

The absorbance of a "reagent blank" must be determined at the start of an analysis to correct for any light absorpton by the solvent or reagents.

Figure 9-2 Block Diagram of a Generic Spectrophotometer



Source: G. H. Schenk, R. B. Hahn, and A. V. Hartkopf, *Introduction to Analytical Chemistry*, 2nd ed., Allyn and Bacon, Boston, 1977. By permission.

Steps in an Analysis

If the analyte is colored, a colorimeter is used for the analysis and the cuvettes can be made of optical glass. If the analyte is not colored, but has an absorption in the ultraviolet, an ultraviolet spectrophotometer is used for the analysis and the cuvettes must be made of quartz or fused silica. In either case, the procedure for an analysis is the same, with the exception of the wavelength region scanned. In the visible region the wavelength range scanned is 760–400 nm, whereas in the ultraviolet region the wavelength range scanned is 400–200 nm.

1. Formation of a Light-Absorbing Species. When a species to be analyzed is not colored and must be analyzed using a colorimeter, it is transformed into a light-absorbing species. (Alternatively, if an ultraviolet spectrophotometer is available and if the species has a functional group that absorbs in the ultraviolet, the use of this instrument may be the easiest way to analyze the sample.) One straightforward way to obtain a colored species is to form a complex. Some metals form highly colored complexes with thiocyanate, SCN^-, for example. A second way to produce a colored species is to transform a metal from a low oxidation state to a higher oxidation state by using an oxidizing agent. Chromium(III), which is only faintly colored, is transformed by oxidizing agents into chromate, CrO_4^{2-} or dichromate, $Cr_2O_7^{2-}$, both of which are intensely colored. Some other types of reactions also produce colored species.

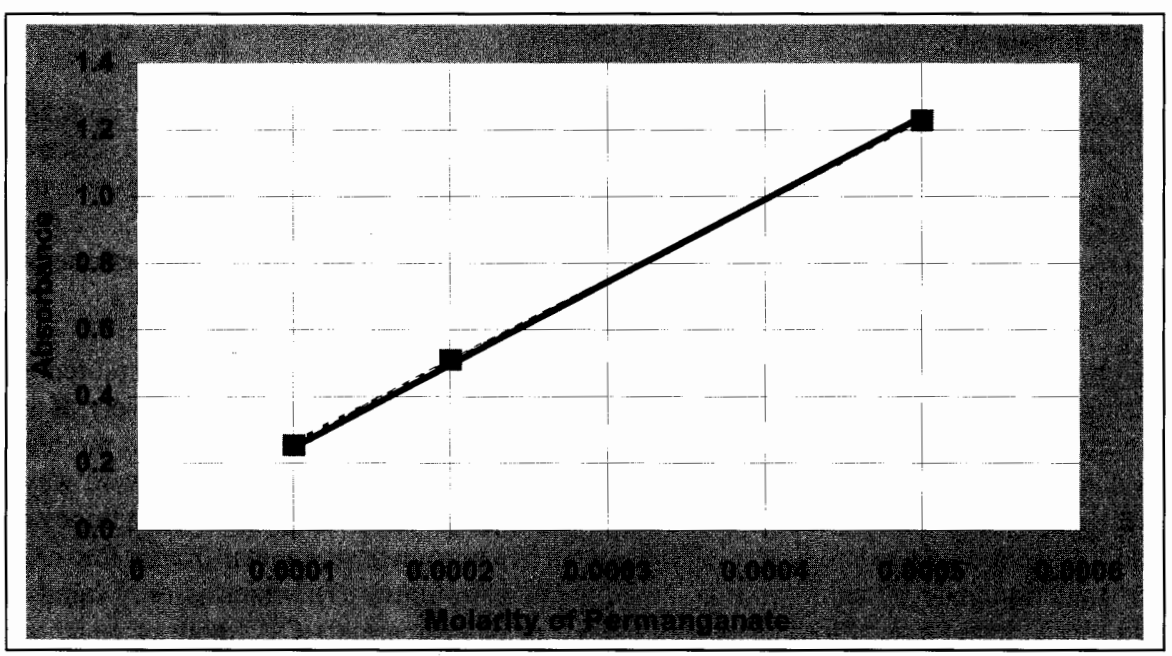
2. Measuring the Absorption Spectrum. The absorbance of the analyte solution is determined as a function of wavelength. The results are plotted (if a recording instrument is not used), preferably using computer software. Ideally, the most intense peak is chosen for the analysis, since it would be the most sensitive to the lowest concentrations. However, if the most intense peak is also sharp, it is better to choose a smaller, broader peak.

The solutions should not contain suspended matter or colloids, which scatter light and distort absorbance measurements.

3. Preparation of a Calibration (Beer's Law) Plot. A series of standard solutions of the analyte is prepared spanning the concentrations expected. The instrument is adjusted to the wavelength chosen for the analysis, and the absorbance of each standard is measured. A plot of absorbance (ordinate) versus concentration is made, preferably using a computer. A least-squares analysis is carried out to obtain the equation of a straight line from which solution concentrations are calculated from measured absorbances. The correlation coefficient from this analysis indicates the precision of the results. Curvature of the plot may indicate a change of equilibrium position of the analyte species with dilution and may have to be taken into account. A Beer's law plot is illustrated in Figure 9-3.

4. Measuring the Sample. The absorbance of the sample is measured at the wavelength used for the calibration. The concentration of the analyte is found from the Beer's law plot (either by estimating directly from the plot or by calculation using the straight-line Beer's law equation). The analyte concentration should be between the extreme limits of the plot; if not, its concentration or the concentrations of the standards should be adjusted accordingly.

Figure 9-3 Beer's Law Plot for Permanganate at 525 nm



Part A: Determining Iron in Natural Waters

Introduction—Iron is found throughout the environment, often in large amounts. It enters the hydrosphere through the weathering of iron salts and minerals. Both iron(II) and iron(III) are found dissolved in water, often in colloidal form, or as inorganic and organic iron complexes. There are many industrial sources of iron, including canneries, tanneries, textile mills, shipping, and metal-cleaning

Large concentrations of iron discharged into a stream or lake may have deleterious effects on aquatic life. A limit of 0.3 mg/L of iron is recommended for food and dairy product processing, soft drink manufacture, and brewing, mainly because of taste.

Iron is a vital element in the respiratory processes of many animals, including humans. The human body has a great demand for iron, and 4 grams are found in the average human. Iron-containing proteins transport oxygen, catalyze the decomposition of peroxides, and play an essential role in the body's energy-generating processes. It is possible to ingest too much iron, which may cause liver damage. Thus, iron vitamin supplements contain cautionary statements.

Theory—A simple but sensitive procedure for the colorimetric determination of iron entails chelating ferrous iron with three molecules of 1,10-phenanthroline (phen) in a solution buffered at low pH,

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

The orange-red complex has an absorption maximum at 510 nm.

A preliminary acid digestion of the sample is carried out in a fume hood to destroy organic matter and also to remove cyanide and nitrate which interfere with the analysis. Hydroxylamine hydrochloride is then added to reduce all iron(III) to iron(II), which is the effective complexing species. Then an excess of phen is added to the sample at a pH between 3.5 and 4.5. The low pH prevents other metals from precipitating and provides rapid reaction and color development. The concentration range of this method is 0.025–3.0 mg/L. Concentrations greater than 3.0 mg/L can be determined after diluting.

An advantage of the 1,10-phenanthroline method is its use of slightly acidic media. This prevents not only the precipitation of hydroxides, but also the phosphates and other anions of many metals. It is necessary that iron be present in a form that reacts completely with 1,10-phenanthroline in a reasonable period of time. This means that iron must not be bound to pyrophosphates or other ligands that form stable complexes; also, phosphate precipitates that contain iron must be prevented from forming. Therefore, the usual procedures in which sodium acetate is used to adjust the pH to 3.5–4.5 are not adequate for biological samples due to the possibility of precipitating ferric and aluminum phosphates. This is avoided by using sodium citrate.

Safety Precautions

1. Safety glasses must be worn at all times in the chemistry laboratory.

2. Use gloves when handling environmental samples.

3. Use extreme care in handling concentrated sulfuric acid. **Wear gloves and goggles.**

Procedure

A: Preliminary Work

1. Attempt to form a complex between thiocyanate ion and iron(II) by mixing together 5 mL each of 0.01 M KSCN and 0.01 M $Fe(NO_3)_2$ (these are provided).

2. Repeat Step 1, this time using 0.01 M $Fe(NO_3)_3$ in acid (also provided) in place of 0.01 M ferrous ion.

3. Use a colorimeter or a recording spectrophotometer to measure the absorption spectrum of the solution prepared in Step 2, scanning from 760 to 400 nm. If necessary dilute the solution to bring its absorbance within the range of the instrument. Use water as the reference.

4. If a Spectronic 20 is used in Step 3, the instrument must first be adjusted to 0%T, at any wavelength. With no sample in the cell compartment, switch the instrument to the Transmittance Mode (if this type of instrument is available; if not, read the %T scale for the following adjustment). Adjust to zero with the front, left-hand knob. Then place a cuvette with water into the cell compartment and use the front, right-hand knob to adjust the instrument to 100%T. This last adjustment must be made each time the wavelength is changed since the intensity of light from the source and the sensitivity of the phototube vary with wavelength.

5. After the adjustments of Step 4, the sample is placed in the cell compartment and its absorbance is read (change the mode to absorbance, if appropriate). If a digital readout instrument is not used, you may wish to make $\%T$ readings and convert them to absorbance via $A = 2 - \log(\%T)$. The rationale for this is that the $\%T$ scale is linear and the absorbance scale is logarithmic, and less error is made in reading the linear scale.

6. Repeat Steps 1, 3, and 4 using Fe(II) nitrate and 5 mL of 0.3% 1,10-phenanthroline solution instead of thiocyanate.

B: Procedure for Iron in Water

1. **Preparation of Standard 100 ppm Iron Solution (this may be provided)** Weigh 351 mg of high quality ferrous ammonium sulfate hexahydrate, $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$, and quantitatively transfer to a 500 mL volumetric flask. Add 50 mL DI water followed by 1 mL of concentrated sulfuric acid. Dilute to the mark with DI water and mix thoroughly.

2. **Preparation of Standard Solutions** Prepare four standard solutions of iron(II) having the following concentrations: 0.5, 1.0, 2.0, and 5.0 ppm. Pipet 0.5, 1.0, 2.0, and 5.0 mL of 100 ppm stock solution into 100 mL volumetric flasks and dilute to the mark with DI water.

3. **Obtaining a Beer's Law Plot** Transfer a 5 mL aliquot of the 0.5 ppm iron standard to a 125 mL Erlenmeyer flask and test the pH with test paper. If greater than 4.5, add enough 0.2 M sulfuric acid dropwise to bring the pH to about 3.5, counting the number of drops. Again counting drops, add sodium citrate (259 g/L) buffer to bring the pH to about 4.5. Pipet 1 mL of 10% hydroxylamine hydrochloride and 3 mL of 1,10-phenanthroline into the sample, mix, and allow 5 minutes for color development.

Use the same number of drops of sulfuric acid and sodium citrate for the remaining three standard solutions, followed by 3 mL of 0.3% 1,10-phenanthroline and 1 mL of 10% hydroxylamine. Mix well.

After adjusting the 0 and 100%T on the colorimeter at 512 nm, use water as the reference and measure the absorbance of each standard. (A reagent blank can also be used if desired. This consists of all substances added to the sample, is treated the same way as the sample, and accounts for any absorbance due to these materials.)

4. **Analysis of Samples** Natural and tap water samples often have less than 0.5 ppm iron. A water faucet that has not been used for some time may furnish a good sample for iron analysis. Some well waters are high in iron content as well. However, even very dilute samples are within the range of this experiment. Determine the iron in several environmental water samples.

Treat samples the same way as the standards, adding sulfuric acid initially, if necessary, followed by citrate buffer, reducing agent and the indicator. Use 5 mL samples and adjust the pH for each sample individually.

Waste Minimization and Disposal

1. Water samples, if they are considered to be hazardous wastes, are disposed of according to the directions of your instructor. Otherwise, they may be flushed down the laboratory drain.

2. Waste ferrous and ferric nitrates can be flushed down the laboratory drain. Follow the instructor's directions for disposing of waste potassium thiocyanate. Do not flush it down the laboratory drain!

Data Analysis

1. Submit the two absorption spectra. From the iron-phenanthroline absorption spectrum decide what wavelength(s) can be used for analysis. Which particular peak, if there is more than one, would be best for an iron analysis?

2. Briefly discuss the effect of the ligand on the wavelength and the maximum absorbance of the peaks.

3. Calculate the extinction coefficient for the largest peak in the absorption spectrum of the iron-phenanthroline complex. (An excess of 1,10-phenanthroline was used.) Assume a cell path length of exactly 1.0 cm. What quantitative information does this provide?

4. Prepare a Beer's law plot for the standard solutions (absorbance as ordinate versus concentration as abscissa). Carry out a least-squares analysis and determine the slope, the y-intercept, and the correlation coefficient for the best straight line; comment on the linearity of the plot.

5. Use your Beer's law plot to determine the concentration (in mg/L) of iron in each sample studied. The concentration of a sample can be obtained directly from the Beer's law plot. Alternatively, the equation of a

straight line can be obtained from the slope and *y*-intercept and the concentration calculated from the sample's absorbance.

If duplicate determinations were done, report average values and the individual values of concentration and discuss the reproducibility.

6. Discuss the magnitude of the iron levels with respect to the sampling site.

7. For the instrument you used, what would you estimate to be the lowest concentration of iron that would be reliable?

Questions and Further Thoughts

1. Where are the major deposits of iron ore in (a) the United States, (b) the Western Hemisphere?

2. Why are elevated levels of iron toxic to humans? What is its major physiological effect?

3. Why is SCN^- not as good as 1,10-phenanthroline in a colorimetric analysis of iron?

Notes

1. The instructor should supervise the handling of concentrated sulfuric acid. A more dilute sulfuric acid solution can be used in place of the concentrated acid.

2. The absorption spectra are best measured with a recording instrument, if one is available.

3. If a Spectronic 20 is used in this experiment, allow at least 30 minutes warm-up time. Keep the instrument on for the entire laboratory period.

Literature Cited

1. J. S. Fritz and G. H. Schenk, *Quantitative Analytical Chemistry,* 5th ed., 331-359 Boston, 1987, pp. 331-359.

2. A. E. Greenberg, L. S. Clesceri, and A. D. Eaton, Eds, *Standard Methods for the Examination of Water and Wastewater,* 18th ed., *American Public Health Association,* Washington, DC, 1992.

3. W. L. Masterton and C. N. Hurley, *Chemistry Principles & Reactions,* 3rd ed., Saunders New York, 1997.

4. D. A. Skoog, D. M. West, and F. J. Holler, *Fundamentals of Analytical Chemistry,7th* ed., Saunders, New York, 1996.