

EXPERIMENT 5

Molecular Absorption Spectroscopy: Determination of Iron with 1,10-Phenanthroline

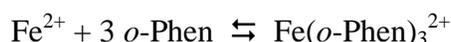
UNKNOWN

Submit a clean, labeled 100-mL volumetric flask to the instructor so that your unknown iron solution can be issued. Your name, section number, and locker number should be written legibly on this flask. The flask does not need to be dry on the inside, but needs to have been rinsed with distilled water after it has been washed. *The flask must be turned in at least 1 lab period before you plan to do the experiment* so that the Teaching Assistants will have time to prepare the unknown. Each student will have his or her own unknown to analyze even if you are working in pairs.

As a backup precaution, bring an MS-DOS formatted floppy disk, or other data-storage medium, so that you can copy the files obtained during the experiment.

BACKGROUND

1,10-phenanthroline (C₁₂H₈N₂, *ortho*-phenanthroline or *o*-Phen) is a tricyclic nitrogen heterocyclic compound that reacts with metals such as iron, nickel, ruthenium, and silver to form strongly colored complexes. This property provides an excellent and sensitive method for determining these metal ions in aqueous solution. For example, *o*-Phen reacts with ferrous ion to produce a deeply colored red complex:



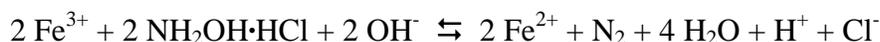
The *molar absorptivity* (ϵ) of the ferrous complex, [(C₁₂H₈N₂)₃Fe]²⁺, is 11,100 L/mol-cm at the wavelength of maximum absorbance intensity, $\lambda_{max} = 508$ nm. This large value indicates the complex absorbs very strongly. The intensity of the color is independent of pH in the range 2 to 9. The complex is very stable and the color intensity does not change appreciably long periods of time. Beer's law is obeyed, over about 1.5-2 orders of magnitude of iron concentration.

Beer's Law is a very simple relationship: $A = \epsilon bc$

where A is the absorbance of a substance at a specified wavelength λ , in units of nm for light in the ultraviolet and visible regions of the electromagnetic spectrum; b is the length of the light path through the sample, usually in cm; ϵ is the molar absorptivity of the absorbing species at λ , when the concentration is in M; and c is the concentration in molar units, M.

The critical point of Beer's Law for use in quantitative analysis is that, theoretically, the absorbance of a solution is linear with the concentration of the absorbing substance, if the wavelength, the pathlength, and other experimental conditions are kept constant. There are a number of factors, however, that limit the concentration range over which Beer's Law is valid for a particular analysis. Perhaps the most critical is concentration. There are invariably deviations from Beer's law at high enough concentrations.

To determine the *total* iron in the sample, it must be completely in the ferrous state, and Fe^{2+} can readily be air-oxidized to the ferric state, Fe^{3+} . *o*-Phen will form a colored complex with Fe^{3+} , but its spectrum is different from that of the ferrous complex and the color is not as intense. Thus, one could not determine the total iron present by making measurements at only one wavelength. Hence, a mild reducing agent is added before the color is developed in order to provide a measure of the total Fe present in solution. Hydroxylamine, as its hydrochloride salt, can be used. The reaction is



This is an equilibrium reaction, but even a mild reducing agent will drive it >99.99% to the right. The pH is adjusted to a value between 6 and 9 by addition of an ammonia or sodium acetate buffer.

INSTRUMENTATION

Varian Cary 50 UV-Vis Spectrophotometer.

This is a computer-controlled double-beam grating spectrophotometer with a constant 20-nm bandpass. A high-intensity Xe flash lamp is used as the source for both UV and visible light, which permits taking 80 data per second.

PREPARATION OF SOLUTIONS

Stock Iron Standard Solution, 10 ppm

Primary standard solid ferrous ammonium sulfate hexahydrate, $(\text{NH}_4)_2(\text{SO}_4)_2\cdot 6\text{H}_2\text{O}$, 392.13 g/mol, is available on the side shelves for preparation of the standard iron solution.

1. Tap a *small* amount of the solid ferrous ammonium sulfate onto a sheet of glassine weighing paper that has been folded in the middle. Zero your balance. *Accurately* weigh about 0.07 g of pure dry ferrous ammonium sulfate (to ± 0.1 mg) onto a folded sheet of glassine paper or into a small, plastic weighing boat.
2. Transfer the ferrous ammonium sulfate *quantitatively* into a 1-L volumetric flask, carefully squirting down the weighing boat and the neck of the flask to ensure a quantitative transfer. Add about 100-200 mL of distilled water. *Dissolve the solid completely before diluting to volume.* This is critical.

3. Pipet 2.5 mL of concentrated sulfuric acid into the flask, rinse the neck of the flask down, and mix carefully with swirling. **[Be very careful when using concentrated H_2SO_4 ; it is quite caustic.]** Dilute the solution to the mark. Calculate the iron concentration of the solution in μg of iron per mL (ppm) and in molar (M) units.

Because this solution is used to calibrate absorbances and prepare a calibration curve, it must be prepared very carefully and accurately. The results of the entire experiment rest on preparing this solution accurately.

The iron solution must be prepared daily, so there is no point in saving the solution to re-use it if you end up needing to re-do the experiment. You will need to prepare another standard solution.

Iron Standard Calibration Solutions

1. Into each of five 100-mL volumetric flasks, pipet 1, 5, 10, 20, and 35 mL of the standard iron solution, respectively. Use a combination of 1-, 5-, 10-, and 25-mL volumetric pipets. The 1- and 5-mL pipets are located in the drawer marked for the experiment.
2. Pour about 50 mL of distilled water into a 6th flask to serve as the “blank” (i.e. zero iron concentration).
3. Obtain the unknown sample from the Teaching assistants and treat it in the same manner as the standards, as indicated below.
4. Line all seven 100-mL volumetric flasks in this order: The blank, those with 1-35 mL of iron stock standard solution added, and your unknown sample. To each flask (including the distilled water “blank” and the unknowns), pipet in order –
 - a. 1 mL of the hydroxylamine solution,
 - b. 10 mL of the 1,10-phenanthroline solution, and
 - c. 8 mL of the sodium acetate solution.

Note that the “blank” solution must have all the reagents in it except for any ferrous ammonium sulfate.

5. Swirl each flask to mix the contents, then carefully dilute each solution to the 100-mL mark and mix thoroughly.
6. Allow the solutions to stand for 10 minutes to fully develop the color. Mix well again. Fill each of seven clean, dry plastic cuvettes about two-thirds full with each of the seven solutions, keeping them in the same order. (If the insides of the cuvettes are wet or spotted, rinse them out twice with the appropriate solution first.)

Stock Reagent Solutions

The sodium acetate buffer (1.2 M), the 1,10-phenanthroline solution (1 g/L), and the hydroxylamine hydrochloride solution (100 g/L) are prepared by the Teaching Assistants and should be available for your use.

PROCEDURE**Determining the Maximum Absorbance of the Complex on the Cary 50**

1. On the Cary WinUV window, select **SCAN**.
2. Click **SET UP** to specify method parameters.
 - a. Set the appropriate mode for the scan in the **X** direction (nanometers).
 - b. Set the wavelength range for the scan by entering 600 nm and 300 nm in the **START** and **STOP** fields.
 - c. In the **Y** field select **ABS** for absorbance.
 - d. Enter 0 and 1.0 for the **Y min** and **Y max** fields.
3. Under **SET UP** select **OPTIONS**.
 - a. Set the beam mode - Select **DUAL**.
 - b. Select **MEDIUM** for scan control.
4. Select **REPORTS**.
 - a. Enter your name in the **NAME** field.
 - b. Enter the sample name in the **COMMENTS** field.
 - c. Select **MAX PEAK** to report the maxima for individual peaks.
5. Insert the blank sample and click **ZERO**.
6. Remove the blank, select one of the iron solutions, and click **SCAN**.
7. When the scan is completed, print 1 copy of the screen per student. *Your lab report should include a printout of the spectrum obtained.*
8. As a backup precaution, copy the spectrum file onto a floppy disk or other storage medium: Click **FILE**, select **SAVE AS**, and save on drive a: with an identifying filename.

Determining the Absorbances of the Test Solutions

1. After scanning the spectrum, select the wavelength of maximum absorption (λ_{max}) to use for the determination of iron with 1,10-phenanthroline.
2. To measure individual standards and your unknown sample, select **SIMPLE READ** in the Cary WinUV menu.
3. Click **SET UP** and select **READ at WAVELENGTH** and enter the wavelength of maximum absorption.

4. In the **Y** mode select **ABS** and click **OK**.
5. Insert the blank sample and click **ZERO**.
6. Measure the absorbance of each of the standard solutions and the unknowns by clicking **READ**.
7. Plot absorbance vs. the concentration of the standards.
8. Note whether Beer's law is obeyed. From the absorbance of the unknown solution, calculate the concentration of iron in the unknown in units of $\mu\text{g/mL}$ and in M units.

LABORATORY REPORT

In addition to other items commonly required in a laboratory report, calculate the concentration of your unknown both (1) by reading the concentration directly off the calibration curve and (2) by using the average of the molar absorptivities (actually ϵb) calculated from each of the five standard solutions. If the plot is linear the values should be the same, and the slope of the absorbance vs. concentration (in M) plot should be equal to ϵb according to Beer's Law: $A = \epsilon bc$. Assuming the pathlength of the cuvette used is 1.00 cm, calculate the molar absorptivity, ϵ , of the ferrous *o*-Phen complex and compare with the literature value of 11,100 L/mol-cm. Comment on the reasons for any difference.

HAZARDOUS WASTE DISPOSAL

Empty ALL the solutions and left over reagents into the proper Hazardous Waste Bottle for this experiment. This includes the blank, unknown, and standard solutions; the remaining stock iron standard solution; small amounts of concentrated sulfuric acid, hydroxylamine hydrochloride, and ortho-phenanthroline solutions that may be left over; and any solid ferrous ammonium sulfate that may be left over from your experiment. If you are unsure of the proper container, ASK.

TEXT REFERENCE

D. A. Skoog, D. M. West, F. J. Holler, and S. R. Crouch, *Analytical Chemistry: An Introduction*, 7th ed., Chapters 21 and 22, pp. 547-592.

Revised September 30, 2005

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