

Experiment 6: Spectrophotometric Determination of Iron in a Vitamin Tablet

Objective: In this experiment, you will use the quantitative technique of spectrophotometry to determine the mass of iron contained in a commercially available vitamin tablet.

Introduction

Iron and the Human Body

The element that is found in the fourth greatest abundance in the earth's crust is also encountered everywhere in biological systems. These two facts about iron are not unrelated as nature has found it logical to adapt this plentiful element to perform a variety of functions. The fact that iron has two readily accessible oxidation states, Fe(II) and Fe(III), also contributes to its usefulness as an active component of proteins.

The average adult human body contains 4-6 g of iron. In human beings, the majority of iron present is found in the blood in a protein called **hemoglobin**. The function of this protein is to transport oxygen from the lungs to the various tissues in the body where it is used to produce energy. One of the byproducts of this metabolism, carbon dioxide, is then transported back to the lungs by hemoglobin.

Humans obtain the iron necessary for the formation of hemoglobin from their diet in foods such as meat and leafy, green vegetables. When the dietary intake is deficient in iron, a condition called anemia results. Someone who is anemic exhibits a lack of energy and often unusually pale skin tone (the red color of blood is also a result of the presence of iron in hemoglobin). Dietary supplements of iron in the form of vitamin tablets can be administered to help alleviate this condition. The tablets you will examine contain iron in the form of ferrous fumarate, $[\text{Fe(II)}(\text{C}_4\text{H}_2\text{O}_4^{2-})]$.

Spectrophotometric Analysis

One of the most common techniques used in the quantitative analysis of samples for a specific chemical substance is called **spectrophotometry**. In spectrophotometry, the amount of electromagnetic radiation absorbed by a sample is measured with an instrument called a **spectrophotometer**, and this absorbance is related to the concentration of the species for which the sample is being analyzed. When a source of visible radiation is used, the relationship between absorbance, A , and concentration, c , is known as **Beer's law**:

$$A = \epsilon c l \quad \text{Eq. 1}$$

In this equation, the concentration is expressed in *molarity*. Molarity is represented by a capital, M (see Tro, pp 152-154):

$$\text{molarity} = M = \frac{\text{number of moles of solute in the solution}}{\text{volume of solution in liters}} \quad \text{Eq. 2}$$

Thus, the number of moles of solute present in a solution can be calculated in the following manner:

$$\text{number of moles solute} = (\text{molarity}) \cdot (\text{volume of solution in liters}) \quad \text{Eq. 3}$$

The other variables in Beer's law are: ϵ , a constant that is a characteristic of the absorbing species called the **molar absorptivity**, and ℓ , the length of the absorption cell that contains the solution. You should read all of Section V. in the Techniques section of the lab manual/notebook to understand fully the instrumentation and principles involved in measuring the absorbance of a solution.

Because the molar absorptivity value is not always a known quantity, a **calibration curve** is often constructed from a series of **standard solutions**. A standard solution is one in which the concentration of the species being analyzed is known. The absorbances of several standard solutions are measured, and these values are plotted as a function of the concentrations of the solutions. If the absorbing species behaves according to Beer's law, such a plot should produce a straight line (see **Eq. 1**). The absorbance of a solution of unknown concentration can then be measured with the spectrophotometer, and this value can be used in conjunction with the calibration curve to determine the concentration of this solution. Read Section IV in the Techniques section to find out more about *straight line graphs* (p. 36) and *Excel* (pp 39-43), especially the *LINEST* function.

Analyzing for iron spectrophotometrically

In this experiment, you will spectrophotometrically analyze a commercially available vitamin tablet in order to determine the quantity of iron that is contained in the tablet. To do this, the iron will first be converted into a form that absorbs radiation in the visible region. This conversion will be done by reacting the iron with an organic compound called 1,10-phenanthroline. The structure of this molecule is shown in Figure 1. It reacts with Fe(II) to form an ionic compound that contains one iron ion and three 1,10-phenanthroline molecules (the structure of this compound is also shown, in Figure 2). The color of this compound in solution is bright red-orange; consequently, it absorbs light very strongly in the visible region at a wavelength of 508 nanometers (see Tro, pp 1144-1145 and also the visible spectrum on page 302).

You will react 1,10-phenanthroline with a vitamin tablet that has been dissolved in hydrochloric acid. In order for this reaction to occur, the iron ions that are present must be in the Fe(II) oxidation state. Since Fe(II) is easily oxidized to Fe(III) in the presence of acid and water, a reducing agent called hydroquinone is added to the solution. In addition, it is important that the acidity of the solution is carefully controlled or compound **1** will not form; the presence of sodium citrate in solution will neutralize some of the acid and maintain the proper pH. The resulting solution will be diluted to an

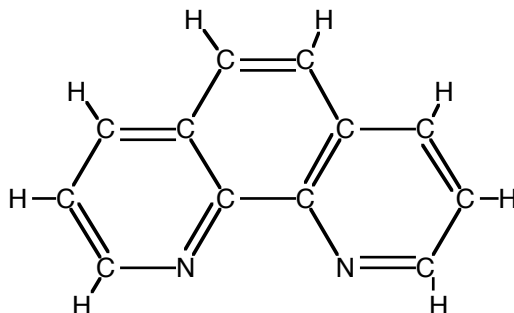


Figure 1. The structure of 1,10-phenanthroline

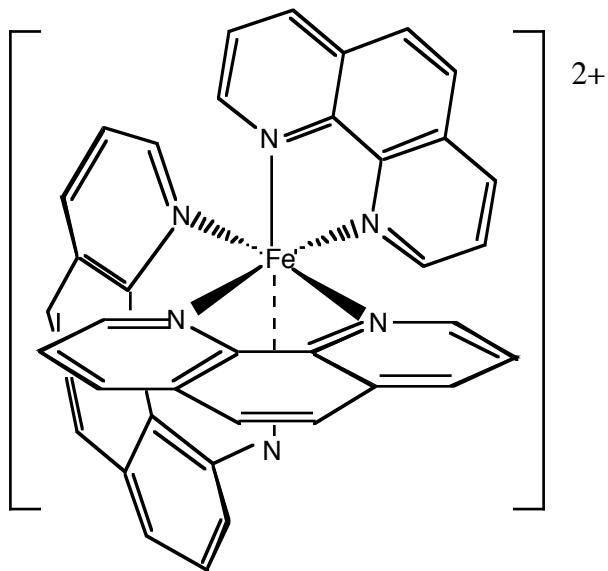


Figure 2. The structure of the iron-1,10-phenanthroline compound, **1**.

appropriate concentration and its absorbance measured. A spectrophotometric calibration curve will then be constructed from a series of standard solutions that contain known concentrations of the iron-1,10-phenanthroline compound (**1**). The concentration of iron in your sample solution, and thus the amount of iron contained in the vitamin tablet, can subsequently be calculated.

Procedure

Preparing the “original” iron solution

Grind a vitamin tablet with a mortar and pestle (it does not have to be finely ground). Obtain 25 mL of 6 M hydrochloric acid in a 150 mL beaker and place the ground vitamin tablet in this solution. Swirl the beaker and wait a few minutes for evidence of a reaction. After the initial reaction has subsided, place the beaker on a hot plate **in a fume hood**, cover with a watchglass and heat to boiling. Boil the mixture for 15 minutes. You may need to add more deionized water during the boiling period if the volume falls below about 15 mL. Remove the beaker from the hot plate and rinse the bottom of the watchglass using a wash bottle, catching the rinse water in the reaction beaker.

Filter the solution while still warm by gravity filtration (see TECH I.G) directly into a 100 mL volumetric flask. Rest the funnel in a wooden funnel holder. While the solution is filtering, heat some deionized water in a 250 mL beaker on a hot plate. When the filtration is completed, use *small amounts* of this hot water to rinse out the beaker, pouring the rinse water on the solid residue contained in the filter paper such that it filters into the 100 mL volumetric flask. Then use small amounts of the hot water to wash the residue two more times. Be careful not to overshoot the mark on the volumetric flask with these rinses. Turn off the hot plate.

Allow the solution in the volumetric flask to cool to room temperature, then dilute to the mark with deionized water (see TECH II.G) and mix. Be sure to add the final amount of water to the volumetric flask with a dropper so that you do not overshoot the calibration mark! This solution in the volumetric flask is what we will call the “**original**” iron solution. Pour this solution into a clean, *dry* 250 mL Erlenmeyer flask, label it with the words “Original Iron Solution” and set aside. Rinse the volumetric flask three times with small portions of deionized water, discarding the rinses into the sink.

The first dilution

Obtain a 5 mL volumetric pipet and rinse it two or three times with small portions of the solution contained in the Erlenmeyer flask (see TECH II.D). Pipet 5 mL of the iron solution into the volumetric flask, dilute to the mark with water and mix. Label the volumetric flask “Flask A”. [Set aside the Original Iron Solution in case you make a mistake during the dilution procedures. When you are finished with the experiment, pour this solution into the **Laboratory Byproducts** jar labeled *Iron + HCl*.]

Determining the amount of sodium citrate required to maintain the proper pH

Obtain a 10 mL volumetric pipet and rinse it two or three times with small portions of the solution contained in Flask A (the volumetric flask). Label a clean, *dry* 125 mL Erlenmeyer flask “Flask B”, then pipet 10 mL of the solution in Flask A into Flask B. Obtain about 8 mL of sodium citrate solution in a graduated cylinder and a strip of indicator paper. Add the sodium citrate solution dropwise to the solution in Flask B, counting the drops as you add them. After you have added 10 drops, test the solution with the indicator paper. The paper should turn yellow-green, indicating that a pH between 3 and 4 has been reached (a color chart will be available for comparison purposes). If a pH *lower* than 3 is indicated, continue to add sodium citrate solution dropwise, checking the solution with the indicator paper after every four or five drops until the proper color is reached. If a pH *higher* than 4 is indicated, start over with a new 10 mL aliquot of the solution from Flask A, and add less than 10 drops of sodium citrate

before testing the pH. Record the total number of drops of sodium citrate that were required to reach a pH of 3 to 4 in your notebook.

The second dilution and conversion of iron to the iron-1,10-phenanthroline compound

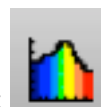
Label a clean, dry 250 mL Erlenmeyer flask "Flask C". Pour the contents of Flask A into Flask C and set aside. Rinse the volumetric flask (Flask A) with deionized water (discard the rinses in the sink). Pipet 10 mL of the solution in Flask C into the volumetric flask. Add the same number of drops of sodium citrate that were required to properly neutralize the solution (the number recorded in your notebook). Then add 2 mL of hydroquinone solution and 3 mL of 1,10-phenanthroline solution to the volumetric flask and swirl. Dilute to the mark with deionized water and mix thoroughly. Let this solution stand for at least 15 minutes before measuring the absorbance. [You may discard the contents of Flasks B and C in the sink.]

Preparing the calibration curve and measuring the absorbance of the sample solution
(you do not need to outline instructions for using the LoggerPro software)

See TECH Section V (pp 44-45) for instructions on how to use a spectrophotometer and especially how to fill a cuvet. **Note:** *never* put acetone into a cuvet as it will "cloud" the plastic. You and one other student will be assigned to use a computer with a SpectroVis Plus (SVP) device. You must measure the absorbances of all the standard solutions using the same SVP, and later you must also use this SVP to measure the absorbance of your own sample solution.

Be careful! If you spill liquid in the SVP, the instrument will no longer function.

In the Applications folder, click on the button labeled **Logger Pro**. You will calibrate the SVP spectrophotometer using deionized water as a blank. From the **Experiment** menu, choose **Calibrate** followed by **Spectrometer: 1**. Wait 90 seconds for the lamp to warm up. Fill a clean dry cuvet with deionized water until the liquid is at the line below the inverted triangle, then place it in the SVP. Be sure the inverted triangle on the cuvet is next to the white triangle that is painted on the box. When the warm-up is completed, click on **Finish Calibration**. When the calibration is completed, click on **OK**.



Click on the **Configure Spectrometer** button in the toolbar:

In the dialog box that appears, click in the circle next to **Absorbance vs. Concentration**. On the left-hand side of the box, type in *Concentration* under **Column Name:**, *Conc* under **Short Name:** and *mol/L* under **Units:**. Scroll through the listing in the center of the box to find the number that is closest to **508 nm**. Click in the appropriate box to place a \checkmark next to this wavelength. Choose **Single 10 nm Band** from the drop-down menu on the left. Click on **OK**.

Each pair assigned to a computer and SVP must obtain the absorbance readings of the standard solutions using their instrument. The standard solutions contain the iron-1,10-phenanthroline compound, **1**, in water at the following concentrations: 0.500×10^{-5} M; 1.00×10^{-5} M; 2.00×10^{-5} M; 3.00×10^{-5} M; 5.00×10^{-5} M

Click on the **green arrow** button. A circular button that is **blue** will appear next to the **red rectangle**. Rinse the cuvet with three small portions of the solution you wish

to measure, then fill the cuvet and place it in the SVP. Watch the absorbance reading that appears in the lower left corner of the screen (below the table). When the absorbance is stable, click on the **blue button**. In the box that appears, type in the value for the concentration of the solution (no units) and click **OK**. Use the letter “e” to designate the exponent. For example, 1.00×10^{-5} can be written: 1e-5. Repeat until you have measured the absorbances for all the standard solutions.

When you have acquired absorbance readings for all of the standard solutions, click on the **red rectangle** to stop the data collection. Save the data to the Documents folder.

Important step! Saving your data:

- Save your data by pulling down the **File** menu and selecting **Save As...**
- In the dialog box that appears, the word **Documents** should be displayed in the drop down menu in the middle of the box. If it is not, scroll through the menu on the left, and choose Documents.
- Next to the words **Save as:**, type over the word “untitled” with the name of your file (example: IronCalibrationLAO). You should include your initials in the name you choose.
- Click on **Save**.
- To find your file later, you will have to go to the **Documents** folder. It should have the suffix .cmbl at the end of the file name.

Open the Excel program. Place your cursor in the first cell (A1) and type the word *Concentration* with the units in parentheses. You can label the next column (B) with the heading Absorbance. Go back to the LoggerPro screen. Click and drag to highlight all the data in both columns of the data table. Once these values are highlighted, select **Copy** from the **Edit** menu. Go back to the Excel screen. Click in cell A2. Select **Paste** from the **Edit** menu, and all the data should appear in columns A and B. At this point, you should save the spreadsheet to the Documents folder.

You need to make a new “blank” solution to calibrate the spectrophotometer before measuring your sample solution. Place one drop of sodium citrate solution, one drop of hydroquinone solution and one drop of 1,10-phenanthroline solution into a cuvet that has been thoroughly rinsed with water. Dilute almost to the top of the cuvet with deionized water, and stir gently with a small stirring rod. Use this solution to calibrate the instrument (under the **Experiment** menu). Rinse and fill the cuvet with your sample solution (the contents of the volumetric flask). Place it in the SVP, and record in your notebook the absorbance value that appears in the lower left corner of the screen.

Pour the final sample solution (in the volumetric flask) and the standard solutions into the **Laboratory Byproducts** jar labeled *Iron-1,10-phenanthroline*. Discard the Original Iron Solution into the **Laboratory Byproducts** jar labeled *Iron + HCl*.

E-mail the Excel spreadsheet that you saved in the Documents folder to yourself. For Calculation 1, you will plot and analyze this data using the LINEST function.

Calculations

1. *Plotting and using the calibration curve*
 - Use the program Excel to plot the calibration curve (see TECH IV, pp 39-43). Follow the instructions to draw the “best fit” line through the data points.
 - Use the LINEST function to determine the slope and y-intercept. Follow the instructions in TECH IV to find the concentration, in molarity, of your sample solution using Excel.
 - Print the plot and data table, and hand them in with your report.
2. *Determining the amount of iron in the tablet*
 - Calculate the concentration of iron in your *original* iron solution (the solution in the volumetric flask that was obtained after the boiling and filtration steps) in molarity. Remember, there are **2** dilution factors! See Tro, pp 154-156 for a discussion of dilution calculations. In this case, the unknown quantity is the initial molarity.
 - Knowing the concentration of your “original” iron solution, you can now calculate the number of milligrams of iron in the vitamin tablet (see **Eq. 3**).

Questions

1. The presence of a dirty fingerprint on the cuvet during measurement of the sample solution resulted in the number of milligrams of iron per tablet being reported incorrectly. Do you think the number reported was too high or too low? Explain why.
2. Is your calibration curve a good example of Beer’s law? Why or why not? Calculate the value for molar absorptivity, ϵ , based on the calibration curve that you prepared. Assume the length of the cell, ℓ , is 1.00 cm. Be sure to include the units!
3. List the important sources of error in this experiment and what effect each would have on the results. Discuss in particular any errors that you may have made.