Spectrophotometric Analysis: Determining the Iron Content in Supplements

OBJECTIVE: The goal of this experiment is to use the quantitative technique of spectrophotometry to determine the mass of iron contained in a commercially available supplement tablet.

SKILLS: Using a spectrophotometer; preparing a standard curve, serial dilutions

EQUIPMENT: SpectroVis spectrophotometer, 5.00 mL volumetric pipettes, 50.00 and 100.00 mL volumetric flasks

SAFETY AND DISPOSAL: Any solution containing the red Fe–1,10-phenanthroline complex should be disposed of in the appropriately labeled container. All other waste can be disposed of down the drain.

INTRODUCTION: Iron is the fourth most abundant element in the earth's crust and is an important component in many biological systems. The fact that iron has two readily accessible oxidation states, Fe^{+2} and Fe^{+3}, 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 protein 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. Iron supplements contain iron in a variety of compounds: ferrous fumarate, ferrous sulfate, and ferrous gluconate. Notice that all of these compounds contain Fe^{+2} (ferrous) because Fe^{+3} can be toxic and is less soluble the Fe^{+2}.

Spectrophotometery

One of the most common techniques used in the quantitative analysis of samples for a specific chemical substance is called **spectrophotometry**. Using this technique, the amount of light absorbed by a sample is measured with an instrument called a **spectrophotometer**, and this absorbance is proportional to the concentration of the species being analyzed. The relationship between absorbance, **A**, and concentration, **c**, is known as the **Beer-Lambert law**:

$$A = \varepsilon l c$$

where l is the **path length,** or the distance the light travels through a sample, usually expressed in cm, and ε is the **molar absorbtivity**, or simply a constant of proportionality. For most experiments, the path length is fixed and thus

the relationship simplifies to:

$$A = kc$$

where k is a constant the needs to be determined experimentally. The concentration can be expressed in any convenient unit such as molarity, mg/mL, parts per million (ppm), etc.

In order to determine the concentration of an unknown solution using this technique, a series of solutions of known concentration, or standards, need to be prepared and analyzed. Using the standards, a **standard** or **calibration** curve is constructed by plotting the absorbance of the standards versus the known concentrations. A line is fit through this data and the resulting equation can be used to convert the absorbance measurement of an unknown to the concentration of the unknown. The concentrations of the standard solutions are chosen to produce a range in absorbance that will include the absorbance of the unknown. If the absorbance of an unknown falls outside the range of the standards, then either the unknown needs to be diluted further (if the absorbance is too high) or a new set of standards need to be prepared with a more appropriate ranges of concentrations (if the absorbance of the unknown is too low).

Analysis of Iron

In this experiment, you will spectrophotometrically analyze a commercially available vitamin supplement 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 by reacting it with an organic compound called **1,10-phenanthroline**. The structure of this molecule is shown in Figure 1. It reacts with Fe^{+2} to form a complex that contains one iron ion and three 1,10-phenanthroline molecules (the structure of this compound is shown in Figure 2). The color of this compound in solution is bright red-orange and it absorbs light very strongly in the visible region at a wavelength of 508 nm.

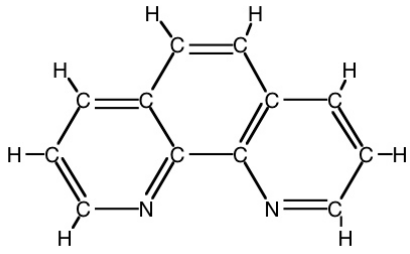

Figure 1: The structure of 1,10-phenanthroline

1,10-phenanthroline will be reacted with a vitamin tablet that has been digested in hydrochloric acid. In order for this reaction to occur to produce the colored complex, the iron ions must be in the Fe^{+2} oxidation state. Since Fe^{+2} is easily oxidized to Fe^{+3} 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 the Fe-1,10-phenanthroline complex will not form. The addition of sodium citrate in solution will neutralize some of the acid and maintain the proper pH.

The resulting solution will be diluted to an appropriate concentration and its absorbance measured. Using data collected from a series of solutions of known concentration a standard curve will be constructed. The concentration of iron in your sample solution, and thus the amount of iron contained in the vitamin tablet, can subsequently be calculated.

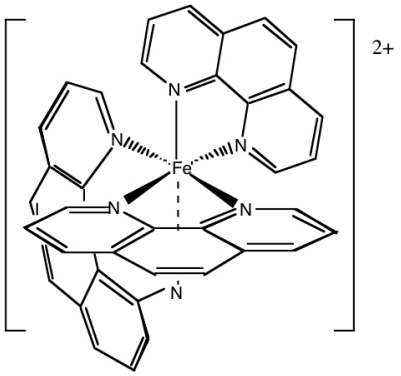

Figure 2: The structure of the iron-1,10-phenanthroline complex

Concentration units and dilutions

Up until this point the main unit of concentration that we have employed has been molarity, or moles per liter. This unit is very helpful in dealing with chemical reactions and stoichiometry. However, there are several additional units that may prove useful in certain situations. For example, in this experiment mass per volume will be used to describe the concentration of solutions. This will enable the determination of the mass of iron in the vitamin supplement tablet. In analytical chemistry the unit of parts per million is also used frequently. For aqueous solutions the conversion factor between ppm and mg/L is: 1 ppm = 1 mg/L.

During the course of this experiment you will need to do several dilution calculations. In general, the following relationship can be applied for dilution calculations with any concentration unit:

$$c_1V_1 = c_2V_2$$

where c represents the concentration of solutions 1 and 2 in the same concentration unit and V represents the volume of solution 1 and 2 in the same volume units. For example, if you take 10 mL of a solution that has an iron concentration of 1 mg/mL and dilute that to a total volume of 100 mL you can determine the concentration of the dilute solution using the above expression since c_1 = 1 mg/mL, V_1 = 10 mL, V_2 = 100 mL, and c_2 is the unknown concentration of the dilute solution that you are solving for. Putting this together and solving for c_2:

$$c_2 = \frac{c_1 V_1}{V_2} = \frac{(1 \text{ mg/mL})(10 \text{ mL})}{100 \text{ mL}} = 0.1 \text{ mg/mL}$$

You will be performing **serial dilutions** during this experiment and you will have to perform this calculation several times.

EXPERIMENTAL PROCEDURE

This is a **quantitative** experiment, so it is very important that you work carefully and consistently throughout. Some general things to keep in mind:

1. Remember that it is good, standard chemical practice to pour small portions of reagents for your use from the stock bottles. Do not risk contaminating the stock bottles by inserting pipettes or other glassware. Also, **NEVER** pour unused reagents back into a stock bottle.
2. For all volume measurements of Fe-containing solutions you must use **volumetric** pipettes. These pipettes are designed to deliver the volume specified and only that volume. When using these pipettes **DO NOT** blow out the last bit of liquid from the tip. Each pipette is calibrated to deliver (TD) exactly the specified volume from the line to where the liquid naturally stops draining.
3. All other reagents are used in excess, so the volumes do NOT need to be measured carefully. You may use clean disposable plastic pipettes or clean graduated cylinders to measure the volumes of other solutions (i.e. that are not Fe-containing).
4. When using a volumetric flask, the specified volume is achieved by having the bottom of the meniscus level with the line on the flask. If the liquid level exceeds the line, that solution must be discarded and prepared again. Therefore, it is useful to add water slowly with a dropper as you approach the line. **You will need three 100 mL volumetric flasks for this experiment.**

The experiment is broken up into three parts: A. preparing your standard curve from the supplied data (done before coming to lab) B. preparing your Fe-tablets for analysis, and C. analyzing your samples using the Spec-20. You need to complete A & B before moving on to using the Spec-20 in part C.

A. Preparation of the standard curve (Pre-lab)

The following standards were prepared and analyzed using the same procedure you will employ in part B. The solutions were prepared from a solution of Fe^{+2} of known concentration. The absorbance of each solution was measured several times and given in the table below.

	Measured Absorbance		
Fe^{+2} Concentration (ppm or mg/L)	**Trial 1**	**Trial 2**	**Trial 3**
0.399	0.081	0.079	0.081
0.799	0.167	0.159	0.165
0.999	0.205	0.197	0.201
1.998	0.410	0.395	0.409
3.996	0.822	0.776	0.817

Before coming to lab, prepare a standard curve using the average absorbance for each concentration. Your standard curve should plot the average absorbance versus concentration on an X-Y scatter plot. You should insert a linear trendline and include the equation of the line & R^2 on your plot. The axes should be labeled correctly. **EACH STUDENT MUST PREPARE HIS OR HER OWN STANDARD CURVE FOR THE PRELAB ASSIGNMENT.**

B. Preparation of vitamin tablet for analysis

You will complete the analysis of two tablets in order to have two independent trials. The most effective way to complete two trials is to perform them in parallel. In doing so, it is important to keep careful track of each solution by labeling all glassware.

Obtain a supplement tablet and record the mass of Fe indicated on the label. Pour 25 mL of 6 M HCl into a clean 150 mL beaker. Add the vitamin tablet and cover with a watch glass. Gently heat the beaker to a boil on a hot plate under your fume hood for 10-15 minutes until the tablet has completely dissolved. Do not allow your samples to dry out. If the liquid level in your beaker is low, add some distilled water from your wash bottle.

After boiling, carefully and quantitatively transfer the solution to a 100 mL volumetric flask. Be sure to rinse the beaker with several small portions of distilled water and pour the washings into the volumetric flask. Once the solution has cooled to room temperature, dilute the solution to the mark with distilled water. **The volumetric flask is calibrated for room temperature measurements, so you must wait for the solution to reach room temperature before diluting to the mark.** Cap the flask and mix well. After mixing, transfer the solution into a clean, dry 250 mL beaker labeled ***Solution 1***.

You will reuse the 100 mL flask from above, so rinse the flask several times with distilled water. Each washing can be discarded down the drain. The next step is to perform a 20-fold dilution of *Solution 1*. Using a 5.00 mL volumetric pipette, transfer 5.00 mL of *Solution 1* into a 100 mL volumetric flask. Dilute the solution to the mark

and mix well. Transfer this solution into a clean, dry 250 mL beaker labeled ***Solution 2***. You will again need to rinse the 100 mL volumetric flask with several portions of distilled water and discard them down the drain as described previously.

Next, you will need to determine the amount of sodium citrate solution necessary to adjust the pH of your solution to the proper level. Using a 5.00 mL volumetric pipette, transfer 5.00 mL of *Solution 2* into a clean 50 mL beaker. Add several drops of bromophenol ble indicator. At this point the indicator should have a yellow color. Add the provided sodium citrate solution dropwise, with stirring, until the red color changes completely to blue/purple. Approximately 40–80 drops will be required depending on your sample. Record the number of drops used. (If you are unsure of the color change that you are looking for, try this step with 5 mL of distilled water first).

The final step is to perform a 10-fold dilution of *Solution 2* while adding the necessary reagents to produce the colored iron complex. Using a 5.00 mL volumetric pipette, transfer 5.00 mL of *Solution 2* into a clean 50 mL volumetric flask. Add the required volume (number of drops) of sodium citrate as determined in the previous step. Next, add 1 mL of hydroquinone and 2 mL of the 1,10-phenanthroline solutions. Dilute to the mark with distilled water , cap, and mix well. Label this ***Solution 3*** and let it stand for 10 minutes to fully develop the color.

Repeat this procedure to prepare a second trial of your selected tablet for analysis. You can label these solutions 1B, 2B, etc.

Preparation of the blank solution

A blank solution (with no colored iron complex) is required to calibrate the spectrophotometer. To reduce waste, a blank was created before the lab and will be available in the laboratory. The blank was prepared by mixing sodium citrate, 2 mL of the hyroquinone and 3 mL of the 1,10-phenanthroline and then diluting to 100 mL with water. Please return this cuvette for the next student to use. Do not discard the blank.

C. Analysis using the SpectoVis Spectrophotometer

You will need to have both trials prepared before proceeding to analyze your samples with the spectrophotometer. In addition, it is helpful to use a small beaker for waste during this part of the analysis.

Before analyzing your samples, the spectrophotometer needs to be calibrated (zeroed) using the blank solution. From the "Experiment" menu select "Calibrate – Spectrophotometer". Allow the lamp to warm up as instructed in the dialog box. Carefully wipe the outside of the cuvette filled with the blank solution so that no fingerprints or smudges, since they would interfere with the reading. Place the cuvette into the spectrophotometer with the clear sides oriented in the direction of the light beam. Follow the on-screen instructions to finish the calibration and then

click “OK”. The instrument is now ready to analyze your unknown samples.

Wash an empty cuvette with a small portion of Solution 3. Pour the washing into your waste beaker. Next, fill the cuvette about ¾ full with Solution 3 and carefully wipe the outside of the cuvette. Place the cuvette into the instrument and press “Collect” to generate the spectrum. Once the spectrum appears, click on “Stop” to end data collection. The autoscale button in the toolbar can be used to adjust the scale. Record the absorbance at 508 nm (the maximum) for your sample by either reading it from the data table on the left of the screen or by using the “Examine” mode button from the toolbar. Note that the absorbance spectrum of your sample is superimposed on the visible spectrum of light. Make a note of what color light is being absorbed by your sample at the maximum in the absorbance spectrum.

Repeat this measurement for your second sample. When prompted, the data from the first trial can be discarded.

When you have completed all of your analyses, enter the mass of iron from the bottle label and the absorbance at 508 nm of each trial on the lab computer.

Calculations: Using the standard curve equation determined in the pre-lab assignment, you can find the concentration of ***Solution 3*** for each trial**.** From this concentration, you can perform two dilution calculations to determine the original concentration of ***Solution 1.*** Next, since you know the total volume of solution (100 mL) and the concentration you can find the total mass of iron in your tablet. Repeat this calculation for each of your trials. Determine the accuracy of your measurements by calculating the relative error, using the mass of iron indicated on bottle label as the “accepted” or “true” value.

Repeat the calculations for the class data available on Blackboard. From the class data determine the average amount of iron in each group of tablets along with the standard deviation.