Colorimetric Determination of Iron in Multivitamins*

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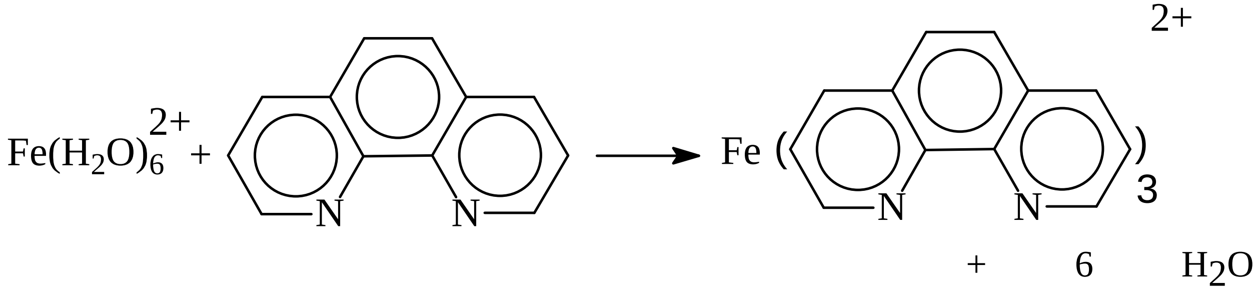
Purpose of this Exercise: to determine the quantity of iron present in multivitamin-mineral dietary supplements by colorimetry. This will require the preparation of a calibration curve using iron solutions of known concentration, dissolution of the multivitamin tablet, dilution to a known volume, and colorimetric comparison of the resulting solution with the calibration curve. We will also analyze the concentration of an iron "unknown" using the same procedure.

Background Information

Iron is an essential nutrient in the human diet. In the body, it is contained in hemoglobin, which carries oxygen from the lungs to the cells of the body, and it plays an equally essential role in respiratory enzymes such as cytochromes which allow us to use the oxygen. We require some dietary iron in order to replace quantities lost by various natural processes, especially bleeding. Principal dietary sources of iron include legumes, eggs, whole grain wheat, oatmeal, and red meat. But many people, even in our well-fed society, suffer from iron deficiency. To avoid such a deficiency, some people take dietary supplements which contain iron. In this exercise, we will determine the amount of iron present in such supplements by a method which is based on an approved method of the Association of Analytical Chemists.

The element iron in water solutions forms salts of Fe^{+2} (ferrous iron) and Fe^{+3} (ferric iron). Ferric iron, however, forms a very insoluble hydroxide, $Fe(OH)_3$, so that ferric salts remain in solution only in very acidic solutions, where the OH^- concentration is very low. In a neutral solution (pH 7), the maximum possible concentration of free Fe^{+3} ions is about 10^{-17} M. While the stomach provides an acidic environment, the intestines (where iron is absorbed) provide a basic environment. Therefore, only ferrous iron can be absorbed and used by the body.

Like other transition metal ions, Fe^{+2} forms many coordination compounds in which the iron ion is surrounded by various Lewis bases, called ligands. Many of these coordination compounds are colored, unlike aqueous ferrous ion itself. If the colored complex is stable and forms quantitatively, we can use the amount of color generated under a standard set of conditions as a measure of the amount of iron present. The ligand used to form a colored complex in this exercise is 1,10-phenanthroline, an organic compound distantly related to ammonia, which forms a stable red complex with **ferrous** iron:



* Adapted from R. C. Atkins, J. Chem. Educ. **52** (1975) 550.

This procedure is selective for Fe^{+2}, even in the presence of other metals, for several reasons. Fe^{+2} reacts rapidly with phenanthroline compared to many other metals, and the complex is exceptionally stable. Most important, it is the only phenanthroline complex which has a strong absorption in the visible spectrum. Therefore, the development of the intense red color due to absorption of light at a wavelength of 508 nm is specifically due to Fe^{+2}, whatever other cations or complexes may be present.

In our preparation of solutions to be analyzed colorimetrically, we will also include two additional reagents: **sodium citrate** is used as a **buffer** to maintain an appropriate pH (3.5) conducive to formation of the complex, and **hydroquinone** is added as a **reducing agent** to intercept oxygen and prevent oxidation of ferrous iron to ferric iron. The procedure to be followed consists of three parts: (a) preparation of a calibration curve by making colorimetric measurements on carefully prepared solutions containing known amounts of Fe^{+2}; (b) preparing a Fe^{+2} solution from a multivitamin tablet and measuring its color with the colorimeter; (c) determining the amount of iron in an "unknown". The solutions we will be using are sufficiently dilute that it is convenient to express the concentrations in mg/L (which is sometimes referred to as parts per million, abbreviated ppm).

Parts 1 and 3 of this exercise may be done in teams of two. Team assignments will be made by your TA. Inform your TA if you wish to prepare the calibration curve without a partner.

Procedures

Part I. Preparation of Calibration Curve

Several solutions for use in performing this exercise will be provided in each laboratory: (a) a 1% solution of hydroquinone in water; (b) a 0.25% solution of 1,10-phenanthroline in water. Since the same amount of these reagents is used in every case, they can be dispensed using Dewick pipettes. The lab will also have (c) a 2.5% solution of sodium citrate in water, which will be dispensed from a buret, and (d) a stock iron solution containing 30-40 mg Fe^{+2} per liter (30-40 ppm). The concentrations will be shown on the label of the container. Be sure to record them in your notebook.

1. Rinse a clean buret, including the tip, with two or three small portions of stock solution of ferrous sulfate. Then fill the buret with this solution.

2. Prepare a blank solution in a clean 100 mL volumetric flask by placing 2.0 mL of sodium citrate solution, 2.0 mL of hydroquinone solution, and 3.0 mL of phenanthroline solution in the flask and diluting to the mark with distilled water, mixing the solution thoroughly. Transfer the blank solution to a labeled container, and reuse the volumetric flask to prepare known solutions. **Save the blank.**

3. Take an initial buret reading, and run about 10.0 mL of the standard iron solution into the 100 mL volumetric flask. Take another reading and subtract readings to determine the actual net volume delivered. Add 2.0 mL of sodium citrate solution, 2.0 mL of

hydroquinone solution, and 3.0 mL of phenanthroline solution. Dilute to the mark with distilled water, and mix thoroughly. This solution is approximately a 1:10 dilution of the stock solution (depending on the exact volume you delivered), so if the stock solution is 40.0 mg/L ferrous ion, this solution is about 4.0 mg/L. Calculate the exact concentration based on the volume you actually used, and label the volumetric flask accordingly.

4. Make four additional standard dilutions by transferring about 7, 5, 3, and 1 mL portions of the stock solution into clean 100 mL volumetric flasks. In each case, determine the precise volume delivered by subtracting buret readings, adding 2.0 mL of sodium citrate solution, 2.0 mL of hydroquinone solution, and 3.0 mL of phenanthroline solution, diluting to the mark with water, and mixing well. These solutions should be clearly labeled. Once each solution has been brought up to volume, it may be transferred to a *clean, dry* beaker so that the volumetric flask can be reused. You may wish to keep the solutions until you have confirmed that you have an appropriate Beer's Law plot.

5. **Allow each solution you prepare to stand for 10 minutes at room temperature, to provide time for formation of the iron-phenanthroline complex.** Measure the absorbance of each solution at a wavelength of 508 nm, using the blank solution to set the 0% absorbance on the spectrophotometer, and recording all data in your laboratory notebook. The absorbance measurements should be done in the order of increasing concentration. The calibration curve, prepared by plotting absorbance vs. concentration for each sample, is to be turned in as part of your report. If computers are used to produce the calibration curve, be sure to enter the data as instructed.

Part II. Determination of Iron Concentration in an Unknown (working alone)

[Since dissolving the multivitamin pill takes some time, you may wish to begin that process before proceeding with the analysis of your unknown.]

1. Your TA will provide you with an unknown solution containing Fe^{+2} in the concentration range 10-100 mg/L. As distributed, this solution is too concentrated to analyze colorimetrically, but we can dilute it and add the necessary reagents just as we did with the stock solution in Part I. Using a pipet, accurately transfer 10.00 mL of your unknown solution to a 100.0 mL volumetric flask. Add 2.0 mL of sodium citrate, 2.0 mL of hydroquinone, and 3.0 mL of phenanthroline solutions (as in Part I). Add distilled water to bring the total volume to the calibration mark of the volumetric flask. **After 10 minutes**, you should determine the absorbance, and from it the concentration. If the absorbance falls outside the range of your calibration curve, make a new dilution as appropriate, again adding the necessary reagents and waiting 10 minutes.

It is recommended that you measure the absorbance of a second sample of the **same diluted unknown solution**. If the absorbance is the same as the first (within the precision of the absorbance measurement), that is evidence both that you waited the appropriate length of time for the formation of the complex and that you mixed the solution to a uniform composition. If the two do not agree, one or the other of the above may not be true. In the latter case, you may wish to prepare a new dilution of your unknown solution.

If time permits, you may wish to prepare a second dilution of your unknown and repeat the above measurements. Report the best value for the initial concentration of Fe^{+2} in the unknown as you received it.

Part III. Vitamin Sample Preparation

1. Record in your notebook the *brand name and description of the tablet you are analyzing*, *including label information on the supposed quantity of iron present*. Put the tablet in a 150 mL beaker, along with 25 mL 6 M. HCl. ***CAUTION*** Boil slowly for 15 minutes in a hood. Do not allow any solution to spatter out of the beaker.

2. Dilute the mixture with 5 mL of water, and **filter** while still hot **directly into** a 100 mL volumetric flask. Wash the beaker, filter, and residue with distilled water, so as to transfer all soluble material into the volumetric flask. Allow the solution in the volumetric flask to cool to room temperature, and add distilled water to bring the volume to the calibration mark of the volumetric flask. This is **Solution X**. (It should be obvious that this solution contains all of the iron in the pill). Pipet a 5.00 mL aliquot of Solution X into a 100 mL volumetric flask, and dilute to the mark with distilled water. This solution, **Solution Y**, now contains one-twentieth of the iron present in the tablet.

3. Pipet a 10.00 mL aliquot of Solution Y into a 50 mL beaker, and determine how much sodium citrate solution must be used to adjust the pH to 3.5. Discard the solution, but use that amount of sodium citrate solution in the following step. This step is necessary because of the variation in types of tablets that may be investigated. Some may require more buffer than others to maintain the appropriate pH.

4. Pipet a 10.00 mL aliquot of Solution Y into a 100-mL volumetric flask, and add the appropriate amount of sodium citrate as determined in step 3, along with 2.0 mL of hydroquinone solution and 3.0 mL of phenanthroline solution. Dilute to exactly 100.0 mL to make **Solution Z**. (Solution Z contains one tenth of the iron in Solution Y and therefore, one two hundredth of the amount in Solution X.) **After 10 minutes**, determine the absorbance of Solution Z. Determine its Fe^{+2} concentration by comparison of its absorbance at 508 nm with your calibration curve. Calculate back to the amount of Fe^{+2} (in mg) in the original tablet, using the Data Sheet to report your results.

5. As with the unknown, measure the absorbance of a second sample of the **same Solution Z**. If the absorbance is the same as the first (within the precision of the absorbance measurement), that is evidence both that you waited the appropriate length of time for the formation of the complex and that you mixed the solution to a uniform composition. If the two do not agree, one or the other of the above may not be true.

Notebook Grade: _____
Safety Grade: _____

SUSB-015 Data Sheet
Determination of Iron Concentration in Unknown

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Name Section Date

Partner's Name______________________________

<u>Beer's Law Data:</u>

Concentration of Iron Stock Solution: _______________mg/L

<u>Solution</u>	**<u>Vol. of Stock Sol'n</u>**	**<u>Conc. of Fe(mg/L)</u>**	**<u>Absorbance, A</u>**
Blank	***_____0_____mL***	***_____0_____.***	***_____0_____***
A	____________mL	____________	____________
B	____________mL	____________	____________
C	____________mL	____________	____________
D	____________mL	____________	____________
E	____________mL	____________	____________

Slope of Beer's Law Plot ___________________

<u>Unknown Determination</u>

	<u>Sample 1</u>	<u>Sample 2</u>
Absorbance of diluted solution	___________	____________
Conc. of Fe^{+2} in diluted solution	___________mg/L	____________ mg/L
Conc. of Fe^{+2} in original solution	___________ mg/L	____________ mg/L

SUSB-015 Data Sheet 2

Determination of Iron Content of Multivitamin Tablet

Description of tablet used, including purported iron ontent:________________________

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	Sample 1	Sample 2
Absorbance of Solution Z	_________	_________
Concentration of Fe^{+2} in Solution Z	_________ mg/L	_______ mg/L
Concentration of Fe^{+2} in Solution Y	_________mg/L	_______ mg/L
Total volume of Solution Y	_________ L	__________ L
Amount of iron contained in Solution Y	_________ mg	_________mg
Amount of iron contained in Solution X = Amount of iron in multivitamin	_________ mg	_________mg

Discuss the agreement between your measurement of the iron content and the purported amount.

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SUSB-015 Pre Lab

Name Section Date

1. (a) The standard recipe used in this exercise uses 3.0 mL of 0.25% phenanthroline solution in 100 mL of final solution to generate the red iron-phenanthroline complex which is determined colorimetrically. What is the initial molarity of phenanthroline in the diluted solution? [0.25% means 2.50 g. of phenanthroline (molar mass 180) per liter of solution.]

(b) What is the maximum concentration of iron-phenanthroline complex which can be formed from this amount of phenanthroline, assuming complete complexation?

(c) What does this concentration of iron correspond to in mg/L?

(d) Is the volume of phenanthroline solution enough to complex the amounts of iron in the unknowns (between 10 mg/L and 100 mg/L)? (Remember that the unknown is diluted before preparing the complex)

2. In dissolving her multivitamin tablet, Lacka Daisickal allows the solution to spatter out of the beaker, causing the loss of about 2 drops (0.10 mL) of the 10 mL of solution that remain after boiling. What error will this cause in her determination of the amount of iron in the tablet?

3. A CHE 134 student, Beavis Butthead, omitted the hydroquinone from the solution he prepared to determine the amount of iron in his unknown. What effect is that omission likely to have on his result?

4. The Beer's Law slope for the Fe-phenanthroline complex is found to be 0.2100 L/mg Fe. The absorbance of the diluted unknown solution prepared in accordance with the instructions in the exercise is found to be X, where X = the last three digits of your USB ID divided by 1000. What is the concentration of the original unknown solution?