

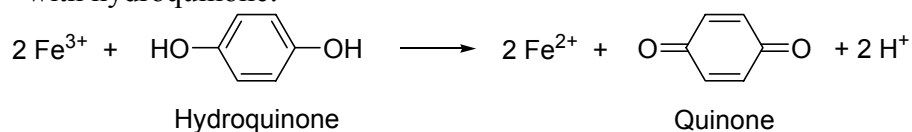
Experiment 2: Spectrophotometric Determination of Iron in Vitamin Tablets

(Adapted from Daniel C. Harris' *Quantitative Chemical Analysis* and R. C. Atkins, *Journal of Chemical Education* **1975**, 52, 550.)

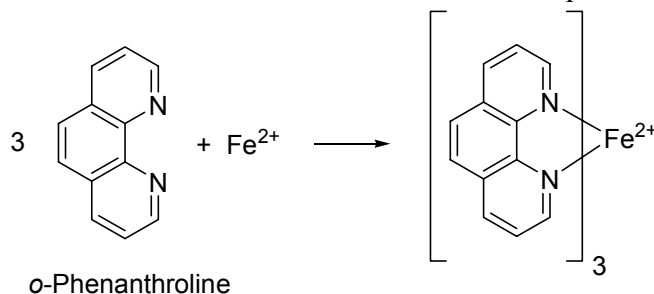
**Experimental Work on February 23 + 30 Minutes on Your Own (in Olin-Rice 387)
Notebook Due on March 3 (by 5 p.m.)**

Introduction

In this experiment, iron from a vitamin supplement tablet is dissolved in hydrochloric acid and then reduced to Fe^{2+} with hydroquinone:



While freshly-dissolved Fe^{2+} in aqueous solution is nearly colorless, we can impart an intense red color by a stoichiometric reaction of Fe^{2+} with three molecules of *o*-phenanthroline (phen):



The complex, which is often written as $\text{Fe}(\text{phen})_3^{2+}$, has a maximum in its absorption spectrum close to 510 nm (the literature value for λ_{max} is 508 nm.) Measuring the solution's absorbance at λ_{max} is a sensitive method of determining Fe concentration.

You will prepare both standard solutions of $\text{Fe}(\text{phen})_3^{2+}$ and a solution with Fe from your vitamin tablet, and measure their absorbances on the Chemistry Department's Beckman DU7400 Spectrophotometer. Construction of a calibration curve will allow you to determine both the molar absorptivity of the $\text{Fe}(\text{phen})_3^{2+}$ complex, and the milligrams of Fe in your vitamin tablet.

Experimental Procedure

A. Basics

- Your TA will have prepared stock solutions of the following reagents, and distributed them throughout the lab:
 - Hydroquinone*: Solution containing 10 g/L in water, stored in amber bottles.
 - Sodium citrate*: 25 g/L in water.
 - o*-Phenanthroline: 2.5 g in 100 mL of ethanol and 900 mL of water, stored in amber bottles. (The ethanol helps dissolve the rather non-polar *o*-phenanthroline.)
 - Stock Fe²⁺ (nominally 0.04 mg Fe/mL)*: Prepare by dissolving 0.5600 g of reagent-

grade $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in water in a 2-L volumetric flask containing 2 mL of 98 wt % H_2SO_4 . We will announce the “official” concentration of the Fe^{2+} solution at the start of lab—do not assume that exactly 0.5600 g of Fe^{2+} reagent was used!

- Remember that it is good, standard chemical practice to pour out small portions of reagents for your team from the stock bottles. Do not risk contaminating the stock bottles by inserting pipets or other glassware. Also, never pour unused reagent back into stock bottles.
- Regarding pipeting,
 - Use the more accurate transfer pipets for all Fe-containing aliquots. The volumes of the other reagent solutions need not be measured very accurately.
 - Use your (brand-new and expensive!) pipet pumps to draw up solutions.
 - Do not blow out the last bit of liquid from a transfer pipet. Each pipet is calibrated to deliver exactly (for example) 10.00 mL from the etched line to where the liquid naturally stops draining.
- You should sign up for a 30-minute time slot to use the spectrophotometer. Everyone should have card access to both Olin-Rice 380 and 387 whenever Olin-Rice is open.

B. Wet Chemistry Procedures

- Place one tablet of an iron-containing vitamin (note which brand you use and the nominal mass of iron per tablet) in a 100-mL beaker and boil gently on a hot plate (in a fume hood) with 25 mL of 6 M HCl for 15 min. Filter the solution directly into a 100-mL volumetric flask using qualitative filter paper. Wash the beaker and filter paper several times with small portions of water to complete a quantitative transfer. If some insoluble bits make it through the filter paper, re-filter your solution before proceeding. Allow the solution to cool, dilute to the mark, and mix well. (Note that is important to let the solution cool before diluting, since volumetric flask marks are accurate only at room temperature.) Dilute 5.00 mL of this solution to 100.0 mL in a fresh volumetric flask. If the label indicates that the tablet contains <15 mg of Fe, use 10.00 mL instead of 5.00 mL. This “twice-diluted” solution is the unknown solution you will use in Steps 4 and 5. While your original unknown solution will likely appear green or yellow (depending on the dyes used in the tablet), your twice-diluted solution will be clear.
- Pipet 10.00 mL of the Fe^{2+} stock solution into a beaker and measure the pH with indicator paper accurate to the nearest pH unit. Add sodium citrate solution 1 drop at a time until a pH of ~3.5 is reached. Count the drops needed. (It will require at least 50 drops.) Don't bother measuring the pH until you've added at least 30 drops. (It may be worthwhile to check the pH of your sodium citrate solution to make sure it is basic!)
- Pipet a fresh 10.00-mL aliquot of the Fe^{2+} stock solution into a 100-mL volumetric flask and add the same number of drops of citrate solution that was required in Step 2. Add 2 mL of hydroquinone solution and 3 mL of *o*-phenanthroline solution, dilute to the mark with water, and mix well by inverting at least 20 times. Then prepare three more standard solutions with 5.00, 2.00, and 1.00 mL aliquots of Fe^{2+} stock solution, and prepare a blank solution containing no Fe^{2+} . All five solutions, including the blank, should contain 2 mL of hydroquinone solution and 3 mL of *o*-phenanthroline solution. (These reagents are in excess, so their volumes do not need to be measured very accurately.) The goal is to make the matrix in all five solutions as similar as possible. However, add sodium citrate solution in proportion to the volume of Fe^{2+} solution. (For example, if 10 mL of Fe^{2+} stock requires 100 drops of citrate solution, 5 mL of Fe^{2+} stock requires 50 drops of citrate solution.) Note any color changes, and any trends in color intensity—do these trends make sense?

4. Take a 10.00-mL aliquot of your twice-diluted unknown solution (which you made in Step 1) and find out how many drops of citrate solution are needed to bring the aliquot's pH up to ~3.5.
5. Transfer a fresh 10.00-mL aliquot of your twice-diluted unknown solution to a 100-mL volumetric flask. Add the required amount of citrate solution determined in Step 4. Then add 2 mL of hydroquinone solution and 3 mL of o-phenanthroline solution; dilute to the mark and mix well.
6. Let the solutions stand for at least ten minutes before making any absorbance measurements.

C. Instrumental Procedure

1. Sign log book.
2. Turn on the spectrophotometer (the power switch is at the back right corner), monitor, and printer.
3. After the instrument has (successfully) completed its power-up diagnostics, use the mouse to click on **Quit** and then on **WAVELENGTH SCAN** (at the upper left of the screen).
4. Click on **VIS OFF** (at the bottom left of the new screen) to turn on the visible light source (a tungsten filament light bulb!).
5. In the upper panel, click next to Start λ to set it to 400 (nm) and click next to End λ to set it to 700.
6. Set the maximum [Abs] value on the y-axis of the spectrum panel to be 1.0.
7. Fill a plastic cuvet (stored in the Styrofoam box) with your blank solution, wipe the smooth sides of the cuvet with a Kimwipe, and place the cuvet in the back of the instrument's sample tray (that is, in Slot 1). Be sure to hold the cuvet by the ribbed sides, and orient the cuvet with the smooth sides exposed to the slits in the side of the tray.
8. Click on **BLANK** in the lower left of your screen. This will store the absorbance in the instrument's memory. Now all subsequent readings will be automatically corrected!
9. Take the blank cuvet out of the sample tray, and replace it with a cuvet filled with your most concentrated Fe^{2+} standard solution.
10. Click on **ReadSamples** (at the upper left of the screen). You should get an absorbance spectrum peaked at around 510 nm.
11. Click on **Print** (upper right) to print out a copy of the spectrum. This should be taped into the notebook your group is using to document your lab work.
12. Click on **Tabulate** (upper left). Scroll down to find the wavelength of maximum absorbance, and write down this λ_{max} and the corresponding absorbance. You will use this λ_{max} for the next part of your measurements. Leave your cuvet filled with your most concentrated Fe^{2+} standard solution in Slot 1.
13. Click on **Exit**, then **Quit** in the upper right of the screen, then **OK**. (There is no need to save a file.)
14. Click on **FIXED WAVELENGTH** (at the upper left of the screen).
15. Click on all three of the wavelength values (to the right of **Sample ID**) and set all of them to the λ_{max} value you determined earlier.
16. Click on the **None** next to **Sampling Device** (in the upper right). Then click on the box next to **Auto smplr** (short for auto-sampler), and set **Number of cells** to 5.
17. Insert into the sample tray cuvetts containing the other three standard solutions and your unknown into Slots 2-5. The front-most cuvet slot (that is, Slot 6) should be empty.

18. Click on **ReadSamples** in the upper left of the screen. The instrument should automatically take three readings on each of the five cuvetts: that is, your four Fe-containing standards and your unknown. Each reading appears in a different column, and all three readings on a given cuvette appear (virtually) simultaneously.
19. Click on **Print** in the upper right of the screen to get a printout.
20. Click on **Quit** in the upper right of the screen, then **OK**. (Again, there is no need to save a file.)
21. In the log book, note if there were any instrumental problems. (Hopefully there weren't!)
22. Turn off the spectrophotometer, monitor, and printer.
23. Rinse out all of your cuvetts several times with deionized water, and leave them to dry next to the sink.

WASTE DISPOSAL: All solutions can go down the drain.

Data Analysis

1. Make a graph of absorbance versus the molarity of Fe (that is, mol Fe/L solution) in the four standards. Plot all twelve points on one graph. Be sure that you have accurately calculated all dilution factors. Use your spreadsheet from Problem Set 3 to calculate the slope (m), y-intercept (b), and the standard errors in the slope (s_m), the y-intercept (s_b), and in an absorbance measurement (s_y) (see Harris Figure 5-9).
2. Using the slope of your calibration curve and the assumption that the cuvette path length is exactly 1.00 cm, calculate the molar absorptivity ϵ of $\text{Fe}(\text{phen})_3^{2+}$ at your λ_{max} . Also report the 95% confidence interval for ϵ . As a check of your work, confirm that it is close to the approximate literature value of $11000 \text{ M}^{-1} \text{ cm}^{-1}$. You do not need to perform a Case 1 t -test for your comparison. However, if your ϵ is, say, a factor of 2 off, you should go back and check your calculations.
3. Use the equation of your calibration curve to determine the molarity x of Fe of the unknown solution whose absorbance you measured.
4. Determine s_x , the standard error in x , in two ways:
 - (a) Propagate the standard errors in slope, y-intercept, and measurement (s_m , s_b , and s_y).
 - (b) Use your spreadsheet to evaluate Equation (5-14) on p. 87 of Harris. Note that $k = 3$ in this formula, since you made three measurements on the unknown.In both cases you should use the s_y value from Equation (5-7) of Harris. Taking the standard deviation of the three absorbance measurements on your sample will underestimate the true uncertainty in y .
5. Convert the molarity determined in Step 3, and the more accurate estimate of s_x determined in Step 4, to units of mg Fe (per tablet). Be sure to keep track of all dilution factors!
6. Perform a Case 1 t -test to see if there is a statistically significant difference (at the 95% confidence level) between your value for mg Fe per tablet and the value on the bottle label. Remember that s_x already includes the factor of $1/\sqrt{n}$. Also note that t_{table} here will be for $12 - 2 = 10$ degrees of freedom, since the precision of our result is determined by the entire calibration curve.
7. Follow the other instructions for lab write-ups I handed out at the start of the semester.
8. Be sure to e-mail me your completed spreadsheet when you turn in your lab notebook.