

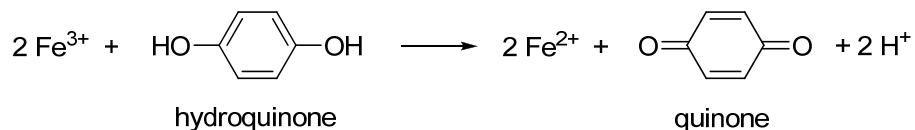
Experiment 2: Spectrophotometric Determination of Iron in Vitamin Tablets

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

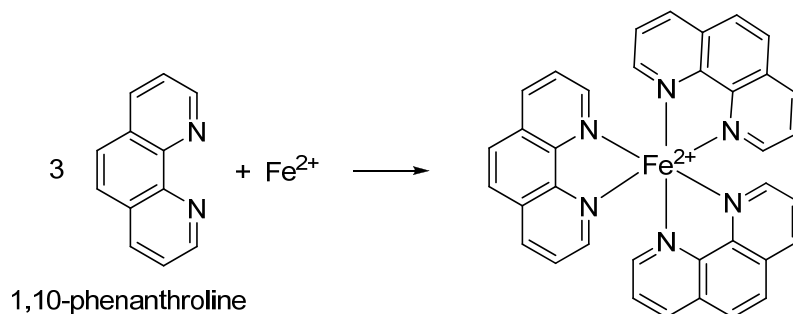
**Experimental work to be done on February 26 + one hour scheduled on your own
Notebook due on March 6 (by 4:00 pm \Rightarrow 20% late penalty each 24 hour period thereafter)**

INTRODUCTION

In this experiment, you will dissolve the iron in a vitamin supplement tablet, digesting the cellulosic matrix in the process, and then reduce the iron to Fe^{2+} with hydroquinone:



While freshly-dissolved aqueous Fe^{2+} is nearly colorless, we can impart an intense red color by a stoichiometric reaction of Fe^{2+} with three molecules of the ligand 1,10-phenanthroline (phen):



The absorption spectrum of the complex, often written as Fe(phen)_3^{2+} , has a maximum at ~510 nm. This complex is stable indefinitely at pH values of 3 or higher. Measuring the analyte solution's absorbance at λ_{max} is a sensitive method for determining iron concentrations.

You will prepare a series of standard solutions containing known concentrations of Fe(phen)_3^{2+} as well as a solution with Fe from a vitamin tablet, and measure their absorbances on the Chemistry Department's Beckman DU7400 spectrophotometer. Construction of a calibration curve using your standard solutions will allow you to determine both the molar absorptivity of the Fe(phen)_3^{2+} complex and, with a pair of measurements of the iron tablet solution's absorbance under the same conditions, the mass of iron that was present in your vitamin tablet.

WASTE DISPOSAL

All solutions from this experiment can go down the drain.

SAFETY

- The hot HCl you are using in this experiment will release corrosive fumes and will rapidly eat into any organic material with which it comes in contact. Wear gloves while working around the hot acid, and heat it only in a fume hood. Of course, wear safety glasses!

- The iron stock solution contains dilute sulfuric acid (H_2SO_4). Unlike HCl, which will evaporate as a gas, sulfuric acid is not volatile. Even a small drop of dilute H_2SO_4 , left out in dry air, will turn into a small speck of much more concentrated sulfuric acid. So clean up any spill with a paper towel; the paper towels used can be safely thrown in the trash. (The H_2SO_4 reacts with the paper, slowly burning it, and neutralizing the acid in the process.)

EXPERIMENTAL PROCEDURE

A. Basics

1. The stock solutions required for this experiment will be provided for you, having been lovingly prepared by your lab instructor and the lab preceptor. They will be as follows:
 - *hydroquinone*: an aqueous solution containing 2 g/L, stored in amber bottles.
 - *sodium citrate*: 50 g/L in water.
 - *1,10-phenanthroline*: 1.0 g/L in water
 - *stock Fe^{2+} (nominally 0.04 mg Fe/mL)*: Prepared by dissolving z g of reagent-grade $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in a 2 L volumetric flask containing 2 mL of 98%_{mass} H_2SO_4 and diluted to the mark with DI water. We will announce the value of z in the Fe^{2+} solution in lab – be sure to write it down, as the actual value will be critical to your calculations!
2. **You must not** contaminate or dilute the stock solutions! Aliquots of the hydroquinone and 1,10-phenanthroline can be poured directly out of their stock bottles, and the sodium citrate dispensed with the provided buret. **Never** insert a pipet or other glassware into a stock bottle, and **never** pour unused reagents back into stock bottles. When pipetting, pour out small portions of the reagent you need from the stock bottles into a beaker.
3. Regarding pipetting,
 - a. *Practice and verify* your pipetting technique, using one of your 10 mL volumetric transfer pipets and your green (10 mL) pipet pump. Confirm that you can reliably transfer 10.0 ± 0.1 g of DI water into a tared beaker on a top loading balance. *Do not use an analytical balance for this.*
 - b. Use the accurate volumetric transfer (not graduated) pipets for all iron-containing aliquots. The volumes of the other solutions need not be measured very accurately.
 - c. If you are having trouble with pipetting, please note the following tips:
 - ii. Use your pipet pumps to draw up solutions. The blue one is for pipets up to 2 mL, the green one for pipets up to 10 mL. They require some practice, but ostensibly should make pipetting a simpler task than it was with the big blue (uncontrolled) pipet bulbs from general chemistry. (Rob is not personally convinced of that, but you can decide for yourself.)
 - iii. Do not blow out the last bit of liquid from a volumetric transfer pipet! Each pipet is calibrated *to deliver* (for example) 10.00 mL of *aqueous* sample from the etched line to where the liquid naturally stops draining, with the tip in contact with clean glass.
 - iv. The liquid level in a pipet will change with the depth to which the tip is immersed in a liquid. When setting the position of the liquid level even with the mark, the tip of the pipet must be *out of the solution and touching a vertical glass surface*. The same needs to be true as it drains, in order to accurately deliver the required volume.

- v. If you need to use a wet pipet, be certain to *rinse* it with whatever you are going to measure with it. Just draw up a pipet full of the liquid you wish to work with and discard it. If the concentration is really critical, you may even wish to do this twice.
4. Keep in mind that we are analyzing for Fe...very low concentrations of Fe. Why is using tap water at any point in this process a really, really bad idea?
5. You should sign up for a one-hour time slot to use the spectrophotometer in the room between the Analytical and Physical Chemistry labs. Everyone should have card access to Olin-Rice 380 whenever Olin-Rice is open, including Saturdays and Sundays.

B. Wet Chemistry Procedures

You should proceed to Step 2 as you wait for aspects of Step 1

1. Thoroughly clean a 100 mL beaker, rinsing it rigorously with DI water. Add 10. mL of DI water to it, then one iron-containing tablet (note the brand you use, and the nominal mass of iron per tablet). Wearing acid gloves, carefully add 10. mL 12 M HCl directly from the repipet into your beaker, then boil gently on a hot plate in a fume hood for 10 min—**but do not boil it to dryness!** [Start the heat setting at 10, then turn it down to 5 as soon as it starts to boil.] Turn off the hotplate, remove the beaker, and once it is cool enough to handle safely, filter the resulting solution directly into a 100.00-mL volumetric flask using qualitative filter paper. Wash the beaker and filter paper several times with small portions of DI water to complete a quantitative transfer. Rinse the extremities of the filter paper with a DI wash bottle until all detectable yellow color has been washed down into the volumetric flask. If any insoluble bits make it through the filter paper, re-filter your solution before proceeding. Allow the solution to cool completely, 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 aliquots of this solution to 100.00 mL in each of two fresh volumetric flasks. If the label indicates that the tablet contains < 20 mg of Fe, use 10.00 mL instead of 5.00 mL; for > 50 mg of Fe, use 2.00 mL instead of 5.00 mL. These “twice-diluted” solutions are the unknown solutions you will use in Step 3. While your original unknown solution will likely appear greenish or yellowish (depending on the dyes used in your tablet), your twice-diluted solutions will most likely be colorless.
2. Pipet 10.00 mL of the Fe²⁺ stock solution into a clean, dry, graduated 100 mL beaker and measure the pH with a pH probe. Add sodium citrate solution 1 drop at a time until a pH of ~3.5 is reached. Count the drops (or note the volume) needed. (It will require at least 15 drops.) *When removing your pH probe from the solution, be sure to rinse it off back into the beaker, so that you don't lose any iron!* Add DI water to bring the volume up to a convenient mark on the beaker, then add 10. mL of hydroquinone solution. (The marks on the beaker are a fine enough guide for this volume.) Next, add 10. mL of 1,10-phenanthroline solution. Quantitatively transfer the contents of this beaker into a 100.00 mL volumetric flask, dilute to the mark with DI water, and mix well by inverting at least 13 times. Then prepare three more standard solutions with 5.00, 2.00, and 1.00 mL aliquots of Fe²⁺ stock solution, and prepare a blank solution containing no Fe²⁺. (These solutions should be made directly in 100.00-mL volumetric flasks.) All five solutions, including the blank, should contain 10. mL of hydroquinone solution and 10. mL of 1,10-phenanthroline solution. (These reagents are in excess, so their volumes need not be

measured with great accuracy.) 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 16 drops of citrate solution, 5 mL of Fe^{2+} stock should require 8 drops of citrate solution.) Note any color changes, and any trends in color intensity – do these trends make sense?

3. Repeat Step 2 with 10.00-mL aliquots of each of your twice-diluted unknown solutions (which you made in Step 1). If you are using a pipet used previously to measure something else, be certain to rinse the pipet with the appropriate twice-diluted solution before using it to transfer volumes of your unknown solution. Remember to bring the solution up to pH 3.5 and add 10. mL of hydroquinone solution and 10. mL of 1,10-phenanthroline solution to each sample before transferring each sample into a clean 100.00 mL volumetric flask; dilute to the mark and invert 13 times. (Your unknown solutions are now thrice-diluted!)
4. Let your solutions stand for at least ten minutes before making any absorbance measurements.

C. Instrumental Procedure

1. Get a beaker from your drawer or the rack in 379 Olin-Rice to use as a waste receptacle.
2. Sign the spectrophotometer log book ("UV log"), located next to the instrument.
3. Turn on the printer, monitor, and spectrophotometer (the power switch is at the back right corner, way at the bottom, way to the rightmost edge), in that order.
4. After the instrument has (successfully) completed its power-up diagnostics, use the left mouse button to click on **Quit** and then on **WAVELENGTH SCAN** (at the upper left of the screen).
5. Click on **VIS OFF** (at the bottom left of the new screen) to turn on the visible light source (a tungsten filament light bulb!) – that should then read **VIS ON**, and turn yellow.
6. 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 650.
7. Set the maximum [Abs] value on the y-axis of the spectrum panel to 1.0.
8. Fill a plastic cuvet (stored in a 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 sample tray.
9. Click on **BLANK** in the lower left of your screen. This will store the blank's baseline absorbance in the instrument's memory, and it will be used to correct your subsequent wavelength scan.
10. Take the cuvet out of the sample tray and pour it out into your waste beaker. Rinse it several times with your most concentrated Fe^{2+} standard solution, being sure to contact any drops of liquid clinging to the inside of the cuvet. Then, fill the cuvet at least half-way full with your most concentrated Fe^{2+} standard solution, and put the cuvet into the sample tray.
11. Click on **ReadSamples** (at the upper left of the screen). You should get an absorbance spectrum peaked at around 510 nm.
12. 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.

13. 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. Write this wavelength in your notebook.
14. Click on **Exit**, then **Quit** in the upper right of the screen, then **OK**. (There is no need to save anything to disk.)
15. Click on **FIXED WAVELENGTH** (at the upper left of the screen).
16. Click on the wavelength value to the right of **Sample ID** and set it to the λ_{\max} value you determined earlier.
17. Insert six cuvetts into the sample tray, each rinsed and filled at least half way with your blank solution. Examine each cuvette before using: if it has a scratch, smudge, or residue on either of the two transparent faces, don't use it; just throw it away. Also, make sure that there are no air bubbles trapped inside any of the cuvetts, at least in the light path.
18. Click on **BLANK** again. This will update the value for the now fully warmed up lamp.
19. Click on **MATCH OFF** to bring up the multiple cuvette blanking window. *Inside this window*, click once on **[MATCH OFF]** in order to change it to **[MATCH ON]**. Click on the Match Wavelength value and change it to the same λ_{\max} value you determined above.
20. Click once on **MatchCells** and wait for the spectrophotometer to strut its stuff. Keep your fingers away from the auto-sampler tray as it moves! The Δ Abs values that appear in the multiple cuvette blanking window should all hover around zero, say ± 0.05 . If any discrepancy is larger than that, check for air bubbles in the cuvette or water drops in the lightpath, and then click **MatchCells** again. When you are done setting up individual blanks for each cuvette, click on **Exit**.
21. To confirm (and document) that you did a good job of blanking out each cuvette properly, hit **ReadSamples**. The six absorbance values obtained should all be 0.000 ± 0.002 .
22. To get an idea of the reproducibility of the instrument, pull out each cuvette, wipe it with the Kimwipe, then stick it back into the sample holder. Once you have done this for all six cuvetts, click on **ReadSamples** again. The values obtained should be very similar to those obtained in the last go-round, but they will likely not be a perfect match. This will give you some idea of how many significant figures the spectrophotometer readings actually deserve. Write down in your notebook what you observe.
23. Now, remove each of the cuvetts from the auto-sampler tray, one at a time, and replace the contents as described in Step 10 above. Be sure to rinse the cuvette with the new solution it is going to contain, and put each one back into the cell holder oriented the *same way* as it was when you ran the blank. *Yes, this can make a difference!* You'll want to charge up the auto-sampler cuvetts with each of your four standard solutions and your two unknowns, placing them into Slots 2-6 as follows: The cuvette slot furthest from you (Slot 1) should contain your most concentrated standard (the one made with 10.00 mL of stock Fe^{2+} solution). Slots 2, 3, and 4 should contain your standards with progressively less iron in them (made with 5.00, 2.00, and 1.00 mL of stock solution, respectively.) Slots 5 and 6 should contain your (replicate) unknown samples.
24. Click on **ReadSamples** in the upper left of the screen. The instrument should automatically take an absorbance reading on each of the six cuvetts. It will automatically correct each reading for the blank measurement specific to the cuvette in question.

25. Click on **Print** in the upper right of the screen to get a printout for your notebook.
26. Click on **Quit** in the upper right of the screen, then **OK**. (There is no need to save a file.)
27. In the log book, note if there were any instrumental problems. (Hopefully there weren't!)
28. Turn off the spectrophotometer, monitor, and printer.
29. Rinse out all of your cuvetts several times with deionized water, and leave them to dry next to the sink, upside down, on a clean paper towel. Thanks!

ANALYSIS

1. Calculate the molarity of Fe^{2+} in the stock solution provided in lab. Show your work in your notebook. Use this result to calculate $[\text{Fe}^{2+}]$ for each of your standard solutions.
2. Make a graph of absorbance versus the molarity of Fe in the four Fe-containing standards. Use Excel's LINEST function to calculate the slope (m), y-intercept (b), and the standard errors in the slope (s_m), y-intercept (s_b), and in a typical absorbance measurement (s_y).
3. Calculate the molar absorptivity, ϵ , of $\text{Fe}(\text{phen})_3^{2+}$ at your λ_{max} . (Assume that the cuvet path length is *exactly* 1 cm.) Also report the 95% confidence interval for ϵ . Confirm that your ϵ is close to the approximate literature value of $1.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. You do not need to perform a Case 1 t -test for your comparison. However, if your ϵ value is, say, a factor of 2 off, you are screwed! (You should go back and check your calculations.)
4. Use your calibration curve equation to find the molarities, x_1 and x_2 , of Fe in the unknown solutions whose absorbances you measured. Determine the average molarity, \bar{x} .
5. Determine s_x , the standard error in your \bar{x} value, in two ways:
 - a. Propagate the standard errors in slope, y-intercept, and measurement (s_m , s_b , and s_y).
 - b. Evaluate Equation (4-27) on p. 71 of Harris. Note that $k = 2$ in this formula, since you made two (mostly) *independent* measurements on the unknown. You'll want to use the average absorbance of your two unknown samples as your y value.
In both cases, use the s_y value from LINEST (rather than taking the standard deviation of replicate absorbance values from your blanks) to determine the uncertainty in y.
6. Convert the average molarity determined in Step 4, and the more accurate estimate of s_x determined in Step 5, to units of mg Fe (per tablet). Keep track of all your dilution factors, and assume they are known exactly. Please note that the relative error s_x/x will be same, whether the ratio is of molarities or mg of Fe in the vitamin pill.
7. Calculate the 95% confidence interval for the mg of Fe in your pill. Clearly state whether or not 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.
8. Do you foresee any potential problems with using this analysis on a multivitamin pill, one containing lots of vitamins or minerals in addition to iron? In analyzing such a pill for iron content, would you expect this technique would work better or worse than the gravimetric approach used in the previous lab? Explain your reasoning.
9. Please tape printouts of your spreadsheet and calibration curve in your notebook, and e-mail the Excel file to me as well.

Please keep in mind the standard Laboratory Notebook Guidelines (as noted in the handout from the start of the semester), including ending with a brief **Conclusion**.