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 24 + one hour scheduled on your own
Notebook due on March 4 (by 4:00 pm ⇒ 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:

\[
2 \text{Fe}^{3+} + \text{hydroquinone} \rightarrow 2 \text{Fe}^{2+} + \text{quinone} + 2 \text{H}^+ 
\]

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):

\[
3 \text{Fe}^{2+} + \text{1,10-phenanthroline} \rightarrow \text{Fe(phen)}_3^{2+}
\]

The absorption spectrum of the complex, often written as Fe(phen)\(_3\)^{2+}, has a maximum at about 510 nm. This complex is stable indefinitely at pH values of 3 or higher. Measuring the analyte solution's absorbance at \(\lambda_{\text{max}}\) 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 preceptors. 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, stored in amber bottles
   - stock Fe^{2+} (nominally 0.04 mg Fe/mL): Prepared by dissolving 0.5602 g of reagent-grade Fe(NH_4)_2(SO_4)_2-6H_2O in a 2 L volumetric flask containing 2 mL of 98% H_2SO_4 and diluted to the mark with DI water.

2. **You must not contaminate or dilute the stock solutions everyone in the lab is relying on!** When pipetting, pour out small portions of the reagent you need from the stock bottles into a beaker. **Never** insert a pipet or other glassware into a stock bottle, and **never** pour unused reagents back into stock bottles.

3. Regarding pipetting,
   - **Practice and verify** your pipetting technique, using one of your 10 mL Class A volumetric transfer pipets and your green (10 mL) pipet pump. Confirm that you can reliably transfer 9.98 ± 0.03 g of DI water into a tared beaker on a top loading balance. **Do not use an analytical balance for this.**
   - 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.
   - If you are having trouble with pipetting, please note the following tips:
     1. Use your pipet pumps to draw up solutions. The blue one is for pipets up to 2 mL, the green one for pipets from 2 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. Use them only to draw **up** reagents, however - disconnect the pump and let gravity drain the pipet.
     2. Do not blow out the last bit of liquid from a volumetric transfer pipet! Each pipet is calibrated **to deliver** its calibrated volume of **aqueous** sample from the etched line to where the liquid naturally stops draining, with the tip in contact with clean glass.
     3. The liquid level in a pipet will change with the depth to which the tip is immersed in 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. No liquid should transfer on the *outside* of the pipet, only the inside.

4. 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, perform this rinse a second time.

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 online for a one-hour time slot to use the spectrophotometer in 379 OLRI, the small instrument room and prep lab space between the Analytical and Physical Chemistry labs. Everyone should have card access to Olin-Rice 380 from 8:00 am to 10:00 pm daily, including Saturdays and Sundays.

**B. Wet Chemistry Procedures**

Digestion and Initial Dilutions of Sample (start working on Preparation of Standards as well)

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).

2. 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 15 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.]

3. 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 ± 0.08 mL (Class A) 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.

4. Allow the solution to cool completely, dilute to the mark, and mix well by inverting at least 13 times. (Note that is important to let the solution cool before diluting, since volumetric flask marks are accurate only at room temperature; glass expands when hot!)

5. Pipet a $y$ mL aliquot of your analyte solution into a fresh 100.0 mL (Class A) volumetric flask. Pipet a second $y$ mL aliquot of your analyte solution into another fresh 100.0 mL volumetric flask. Dilute both flasks to the mark and mix well. So, what’s $y$?

<table>
<thead>
<tr>
<th>Nominal Amount of Fe in Tablet</th>
<th>Aliquot Volume ($y$) to Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 20 mg</td>
<td>10.00 mL</td>
</tr>
<tr>
<td>Between 20 mg and 50 mg</td>
<td>5.00 mL</td>
</tr>
<tr>
<td>Greater than 50 mg</td>
<td>2.00 mL</td>
</tr>
</tbody>
</table>

The “twice-diluted” analyte solutions you have prepared in this step are the unknown solutions you will use in Step 10. (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.)

**Preparation of Standards**
6. Pipet 10.00 mL of the Fe$^{2+}$ stock solution into a clean, dry, graduated 100 mL beaker. Be sure to rinse the pipet with the stock solution before using it. Measuring the pH of this solution and gently stirring it with a pH probe, add sodium citrate solution, 1 drop at a time, until a pH of about 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!**

7. Add DI water to bring the volume up to a convenient mark on the beaker, then add 10. mL of hydroquinone solution and 10. mL of 1,10-phenanthroline solution. (The marks on the beaker are an accurate enough guide for these volumes, as both reagents are in excess.)

8. Quantitatively transfer the contents of this beaker into a 100.0 mL volumetric flask, dilute to the mark with DI water, and invert 13 times to mix well.

9. 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 four solutions should be made directly in 100.0 mL volumetric flasks.**

   1. Pipet the required volume of Fe$^{2+}$ stock solution directly into a 100.0 mL volumetric flask. *Be certain to rinse the pipet with the stock solution first.*
   2. 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.) *Do not measure the pH.*
   3. Add 10. mL of hydroquinone and 10. mL of 1,10-phenanthroline to each of the four volumetric flasks. (These volumes need not be measured with great accuracy.)
   4. Note any color changes and trends in color intensity – do these trends make sense?

**Final Sample Dilution**

10. Pipet 10.00 mL of the first of your twice-diluted unknown solutions into a clean, dry, graduated 100 mL beaker. *Be sure to rinse the pipet with the twice-diluted solution first.* Measuring the pH of this solution with a pH probe, add sodium citrate solution, 1 drop at a time, until a pH of about 3.5 is reached. Count the drops (or note the volume) needed. **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!**

11. Add DI water to bring the volume up to a convenient mark on the beaker, then add 10. mL of hydroquinone solution and 10. mL of 1,10-phenanthroline solution.

12. Quantitatively transfer the contents of this beaker into a 100.0 mL volumetric flask, dilute to the mark with DI water, and mix well. Congratulations: you now have a thrice-diluted unknown solution!

13. Prepare a second thrice-diluted unknown solution as follows:

   1. Transfer a 10.00 mL aliquot of the other twice-diluted unknown solution directly into a 100.0 mL volumetric flask, rinsing the pipet with the unknown solution, first.
   2. Add the same amount of sodium citrate solution that you added in Step 10. *Do not measure the pH again.*
   3. Add 10. mL of hydroquinone solution and 10. mL of 1,10-phenanthroline solution
   4. Dilute to the mark with DI water and invert 13 times to mix.

You now have two (semi-independently prepared) thrice-diluted unknown solutions.
C. Instrumental Procedure (Do not write down what you do or observe here in your notebook, unless the instructions explicitly tell you to do otherwise.)

Finding $\lambda_{\text{max}}$

1. Let your solutions stand for at least ten minutes before making any absorbance measurements. They require that time to fully equilibrate.
2. Get a beaker from your drawer or the rack in 379 Olin-Rice to use as a waste receptacle.
3. Sign the spectrophotometer log book ("UV log"), located next to the instrument.
4. Turn on the monitor, the spectrophotometer, and the printer. After the spectrophotometer has (successfully) completed its power-up diagnostics, use the left mouse button to click on Quit and then on WAVELENGTH SCAN (in the upper left corner of the screen].
5. Click on VIS OFF (in the bottom left corner of the screen) to turn on the visible light source (a tungsten filament light bulb!). It should change to VIS ON and turn red.
6. In the upper panel, click next to Start $\lambda$ to set it to 400 (nm), and then click next to End $\lambda$ to set it to 650 nm.
7. Set the maximum [Abs] value on the y-axis of the spectrum panel to 1.0000.
8. Select a plastic cuvet (stored in a Styrofoam box) that has no scratches, smudges, or residues on either of the two transparent faces. Fill it half-way with your blank solution. Make sure that there are no air bubbles trapped inside.
9. 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, so you don't get your fingerprints on the sample windows, and orient the cuvet with the smooth sides exposed to the slits in the sides of the sample tray.
10. Click on BLANK in the lower left corner of the 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.
11. 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 it half-way with your most concentrated Fe$^{2+}$ standard solution, and put the cuvet into Slot 1 of the sample tray in the same orientation that you used for the blank solution. Yes, this can make a difference! (You may want to mark one side of the cuvet with a Sharpie to help you keep track of orientation; you want to put it into the tray oriented the same way every time.)
12. Click on ReadSamples (in the upper left corner of the screen). You should get an absorbance spectrum with peak at around 510 nm.
13. 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.
14. Click on Tabulate (upper left). Scroll down (click on $\downarrow$) to find the wavelength of maximum absorbance. You will use this $\lambda_{\text{max}}$ for the next part of your measurements. Record this wavelength value in your notebook.
15. Click on Exit, then Quit in the upper right corner of the screen. You may need to unselect a box next to one of the Save options. Finally, click on OK. (There is no need to save anything to disk, but if you do, it's no biggie; it'll just be on a RAM [memory] disk.)
Blanking Your Cuvets

16. Click on **FIXED WAVELENGTH** (in the upper left corner of the screen).

17. Click on the first wavelength value immediately to the right of **Sample ID** and set it to the \( \lambda_{\text{max}} \) value you determined earlier. (Ignore the other two columns.)

18. Click on the 0.50 value next to **Read Average time:** and change the value to **2.00** seconds.

19. Select six plastic cuvets that have no scratches, smudges, or residues on either of the two transparent faces. Rinse them with your blank solution and then fill them half way with your blank. Make sure that there are no air bubbles trapped inside any of the cuvets.

20. Click on **BLANK** again. This will update the value for the now fully warmed up lamp.

21. Click next to **Sampling device:**, where it currently says "None", and choose **Auto smplr**, making sure that the **Number of cells** is set to **6**. Then click OK.

22. Click on **MATCH OFF** (in the lower left corner) to bring up the multiple cuvet 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 \( \lambda_{\text{max}} \) value you determined above.

23. 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! This function does two things: (1) It records a separate blank value for each cuvet. (2) It compares the absorbances of the cuvets in Slots 2-6 to that of the cuvet in Slot 1; the differences are the \( \Delta \text{Abs} \) values. It is not necessary for the \( \Delta \text{Abs} \) values to be exactly zero, but they should be within, say, \( \pm 0.05 \) of zero. If any discrepancy is larger than that, check for air bubbles in the cuvet or water drops in the light path, and then click **MatchCells** again. When you are done setting up individual blanks for each cuvet, click on **Exit**.

24. To confirm (and document) that you did a good job of blanking out each cuvet properly, hit **ReadSamples**. The six absorbance values obtained should all be \( 0.000 \pm 0.002 \). (If not, life goes on; don't spend too much time trying to fix this, beyond perhaps repeating steps 20 to 22 one time. But recognize that there may be greater error for a cuvet whose blank reading is not \( 0.000 \pm 0.002 \).) You don't need to record these values in your lab notebook, as you will be printing them out a bit later on.

Measuring Your Standards and Unknown

25. Remove each of the cuvets from the auto-sampler tray, one at a time, and replace the cuvet contents with either one of your four standard solutions or one of your two thrice-diluted unknown solutions (as specified immediately below). Rinse each cuvet several times with the new solution it is going to contain, being sure to contact any drops of liquid clinging to the inside of the cuvet, and then put it back into the cell holder oriented the **same way** as it was when you ran the blank.

1. The cuvet slot furthest from you (Slot 1) should contain your most concentrated standard (the one made with 10.00 mL of stock \( \text{Fe}^{2+} \) solution).

2. 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.)

3. Slots 5 and 6 should contain your (replicate) thrice-diluted unknown samples.

26. Click on **ReadSamples** in the upper left of the screen. The instrument should automatically take an absorbance reading on each of the six cuvets. It will automatically correct each reading for the blank measurement specific to the cuvet in question.
27. Take each cuvet out of the auto-sampler tray, wait for a minute, and place them back into the tray in the same slot and the same orientation. Then click on ReadSamples again.

28. Repeat Step 27.

[Why in the world are we doing Steps 27 and 28?!? It turns out that two of the biggest sources of random error in a spectrometer are the imprecision of cuvet orientation and drift in the electronics. By taking out and reinserting your cuvets twice, and by waiting a little while between measurements, you will get a good estimate of the combined magnitude of these errors.]

29. Click on Print in the upper right of the screen to get a printout of all the absorbances, for your notebook.

30. Click on Quit in the upper right of the screen, then OK. (There is no need to save a file.)

31. In the log book, note if there were any instrumental problems. (Hopefully, there weren’t any!)

32. Turn off the monitor, spectrophotometer, and printer.

33. Rinse out all of your cuvets several times with deionized water, and leave them to dry next to the sink, upside down, on a clean paper towel. Thanks!
ANALYSIS

1. Plot all 12 standard measurements on a graph of absorbance versus the molarity of Fe, and fit them with a linear trendline that displays the equation of the line and the R² value of the fit on the plot. 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 \( s_y \). Do not plot the blank measurements; your data is already corrected.

2. Use the slope of your calibration curve to calculate the molar absorptivity, \( \varepsilon \), of Fe(phen)\textsuperscript{3+} at your \( \lambda_{\text{max}} \). (Assume that the cuvet path length is exactly 1 cm.) Report the 95% confidence interval for \( \varepsilon \). Use a Case 1 t-test to see if you can be 95% certain that your experimental \( \varepsilon \) does not agree with the literature value\textsuperscript{1} of \( 1.15 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1} \). (If your \( \varepsilon \) value is more than 10% off, you are hosed! Well, not really, but you do need to go back and re-check your molarity calculations.)

3. Calculate \( \bar{y}_{\text{unknown}} \), which is the average of the six absorbances you measured for your two unknown solutions. Use your calibration curve equation to convert \( \bar{y}_{\text{unknown}} \) to the unknown molarity, \( \bar{x}_{\text{unknown}} \).

4. Determine \( s_x \), the standard error in your \( \bar{x}_{\text{unknown}} \) value, in two ways:
   1. Propagate the standard errors in slope, y-intercept, and measurement \( (s_m, s_b, \text{ and } s_y) \), assuming that these errors are all independent of one another.
   2. Evaluate Equation (4-27) on p. 71 of Harris. Please use \( k = 6 \) in this formula, that is, we will pretend we made six truly independent measurements on the unknown. (Do you see that we didn't?) You'll want to use \( \bar{y}_{\text{unknown}} \) as your \( y \) value.

   In both cases, use the \( s_y \) value from LINEST (rather than using the standard deviation in \( \bar{y}_{\text{unknown}} \)).

5. Convert \( \bar{x}_{\text{unknown}} \) and the more accurate estimate of \( s_x \) 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. (If you are not within a factor of two of the nominal value of mg Fe in your tablet, you have probably made a mistake with one or more of your dilution factors. Be careful!)

6. 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. Before you potentially freak out, know this: the FDA basically lets "nutritional supplement" manufacturers set their own quality control rules, so long as they provide at least what they claim on the bottle...so they tend to overshoot the mark.

7. Answer this question: Do you foresee any potential problems with using our approach in Experiment 2 on a multi-vitamin pill which contains lots of vitamins and minerals in addition to iron? In analyzing such a pill for iron content, would you expect the Experiment 2 approach to work better or worse than the gravimetric approach used in the previous lab? Explain your reasoning.

8. Please tape printouts of your spreadsheet and calibration curve in your notebook. Each printout page should be taped to a separate notebook page. (This makes it a lot easier for me to write comments on your printouts.)

9. Please e-mail me your Excel file.

Please remember to follow my "Lab Notebook Guidelines" (from the handout from the start of the semester), including ending with a brief Conclusion.

\textsuperscript{1}Skoog, Holler, and Nieman, Principles of Instrumental Analysis, 5\textsuperscript{th} Ed, Harcourt Brace: Philadelphia (1997).