Colorimetric Determination of Iron

To become acquainted with the principles of colorimetric analysis.

**Apparatus**
- balance
- 125-mL Erlenmeyer flask
- spectrophotometer

**Chemicals**
- standard iron solution, Fe(NO₃)₃
- HNO₃ (1 mL = 0.050 mg Fe)
- 0.30% α-phenanthroline
- unknown iron sample
- 1 M NH₄C₂H₅O₂
- 10% hydroxylamine hydrochloride
- 6 M H₂SO₄

**OBJECTIVE**

To determine the concentration of iron in an unknown solution by colorimetric analysis.

**APPARATUS AND CHEMICALS**

Prepare your calibration curve in teams of three, but analyze your unknown individually.

The basis for what the chemist calls **colorimetric analysis** is the variation in the intensity of the color of a solution with changes in concentration. The color may be due to an inherent property of the constituent itself—for example, MnO₄⁻ is purple—or it may be due to the formation of a colored compound as the result of the addition of a suitable reagent. By comparing the intensity of the color of a solution of unknown concentration with the intensities of solutions of known concentrations, the concentration of an unknown solution may be determined.

You will analyze for iron in this experiment by allowing iron(II) to react with an organic compound (α-phenanthroline) to form an orange-red complex ion. Note in its structure (shown below) that α-phenanthroline has two pairs of unshared electrons that can be used to form coordinate covalent bonds.

The equation for the formation of the complex ion is:

\[
3C_{12}H_8N_2 + Fe^{2+} \rightarrow [(C_{12}H_8N_2)_2Fe]^{2+}
\]

**DISCUSSION**

Unshared electron pairs

α-phenanthroline
Before the colored iron(II) complex is formed, however, all the Fe$^{3+}$ present must be reduced to Fe$^{2+}$. This reduction is accomplished by the use of an excess of hydroxylamine hydrochloride:

$$4\text{Fe}^{3+} + 2\text{NH}_2\text{OH} \rightarrow 4\text{Fe}^{2+} + \text{N}_2\text{O} + 6\text{H}^+ + \text{H}_2\text{O}$$

Hydroxylamine

Although the eye can discern differences in color intensity with reasonable accuracy, an instrument known as a spectrophotometer, which eliminates the “human” error, is commonly used for this purpose. Basically, it is an instrument that measures the fraction $I/I_0$ of an incident beam of light of a particular wavelength and of intensity $I_0$ that is transmitted by a sample. (Here, $I$ is the intensity of the light transmitted by the sample.) A schematic representation of a spectrophotometer is shown in Figure 31.1. The instrument has these five fundamental components:

1. A light source that produces light with a wavelength range from about 375 to 650 nm
2. A monochromator, which selects a particular wavelength of light and sends it to the sample cell with an intensity of $I_0$
3. The sample cell, which contains the solution being analyzed
4. A detector that measures the intensity, $I$, of the light transmitted from the sample cell; if the intensity of the incident light is $I_0$ and the solution absorbs light, the intensity of the transmitted light, $I$, is less than $I_0$
5. A meter that indicates the intensity of the transmitted light

For a given substance, the amount of light absorbed depends on the

1. Concentration
2. Cell or path length
3. Wavelength of light
4. Solvent

Plots of the amount of light absorbed versus wavelength are called absorption spectra. There are two common ways of expressing the amount of light absorbed. One is in terms of percent transmittance, %T, which is defined as

$$%T = \frac{I}{I_0} \times 100$$

[1]
As the term implies, percent transmittance corresponds to the percentage of light transmitted. When the sample in the cell is a solution, I is the intensity of light transmitted by the solution, and I₀ is intensity of light transmitted when the cell only contains solvent. Another method of expressing the amount of light absorbed is in terms of absorbance, A, which is defined by

$$A = \log \frac{I_0}{I}$$

[2]

The term optical density, OD, is synonymous with absorbance. If there is no absorption of light by a sample at a given wavelength, the percent transmittance is 100, and the absorbance is 0. On the other hand, if the sample absorbs all of the light, %T = 0 and A = ∞.

Absorbance is related to concentration by the Beer-Lambert law:

$$A = abc$$

where A is absorbance, b is solution path length, c is concentration in moles per liter, and a is molar absorptivity or molar extinction coefficient. There is a linear relationship between absorbance and concentration when the Beer-Lambert law is obeyed, as illustrated in Figure 31.2. However, since deviations from this law occasionally occur, it is wise to construct a calibration curve of absorbance versus concentration.

### A. Preparation of the Calibration Curve

Accurately pipet 1.00 mL of standard iron solution (1.00 mL = 0.050 mg Fe) into a 50-mL volumetric flask. Add 1 mL of 1 M ammonium acetate, 1 mL of 10% hydroxyamine hydrochloride, and 10 mL of 0.30% o-phenanthroline solution. Dilute to exactly 50.0 mL with distilled water. Mix well to develop the characteristic orange-red color of the iron(II)-phenanthroline complex. Allow the color to develop for 45 min. Fill halfway a clean, dry cuvette (colorimeter tube) with the colored solution and determine the absorbance at 510 nm using a Spectronic 20 or other colorimeter. Operating instructions for the Spectronic 20 are given below.

Repeat using 2.0-mL, 3.0-mL, 4.0-mL, and 5.0-mL portions of the standard solution. Plot your results with milligrams of iron along the abscissa.

**PROCEDURE**
Instructor: Explain the operation of the spectrophotometer that the students will use.

![Spectrophotometer controls](image)

**FIGURE 31.3** Spectrophotometer controls.

(horizontal axis) and absorbance along the ordinate (vertical axis). This curve should be turned in with your report sheet. (HINT: Time may be saved if these solutions are all made at the same time.)

### Operating Instructions for Spectronic 20

1. Turn the wavelength-control knob (Figure 31.3) to the desired wavelength.
2. Turn on the instrument by rotating the power control clockwise and allow the instrument to warm up about 5 min. With no sample in the holder but with the cover closed, turn the zero adjust to bring the meter needle to zero on the “percent transmittance” scale.
3. Fill the cuvette about halfway with distilled water (or solvent blank) and insert it in the sample holder, aligning the line on the cuvette with that of the sample holder; close the cover and rotate the light-control knob until the meter reads 100% transmittance.
4. Remove the blank from the sample holder and replace it with the cuvette containing the sample whose absorbance is to be measured. Align the lines on the cuvette with the holder and close the cover. Read percent transmittance or optical density from the meter.

### B. Determination of Iron

Accurately weigh out about 0.1 g to four significant figures (0.05 g for 12% to 15% iron samples) of the iron unknown into a 50-mL volumetric flask, add 5 drops of 6 M sulfuric acid, and dilute to exactly 50 mL. Mix thoroughly and then transfer this solution to a clean 125-mL Erlenmeyer flask. Pipet exactly 1 mL of this solution into a thoroughly rinsed 50-mL volumetric flask and repeat the procedure described above in Part A (do not add standard iron solution). Repeat this procedure on two additional 1-mL aliquots of unknown solution.

Using the observed absorbance and the calibration curve, calculate the milligrams of iron in 1 mL of solution and the percentage of iron in the sample. Calculate the mean and standard deviation of your results.

**Waste Disposal Instructions** Sulfuric acid and the organic solutions are hazardous. Do not pour the solutions down the drain of the sink. Use the appropriate containers in the laboratory for disposal.
Before beginning this experiment in the laboratory, you should be able to answer the following questions:

1. For a given substance, the amount of light absorbed depends on what four factors?
2. How are percent transmittance and absorbance related algebraically?
3. What are the five fundamental components of a spectrophotometer?
4. State the Beer-Lambert law and define all terms in it.
5. What is the purpose of preparing a calibration curve?
6. Why is hydroxylamine hydrochloride used in this experiment?
7. If the percent transmittance for a sample is 100 at 350 nm, what is the value of $A$?
8. Suppose your experimental absorbance is greater than 1. How would you modify your procedure?
9. If 3.0 mL of a standard iron solution (1 mL = 0.050 mg Fe) is diluted to 50 mL, what is the final iron concentration in mg Fe/mL?
10. If aqueous Co(NO$_3$)$_2$ has an extinction coefficient of 5.1 L/mol-cm at 505 nm, show that a 0.0875 M Co(NO$_3$)$_2$ solution will give an absorbance of 0.45.
REPORT SHEET | EXPERIMENT

Colorimetric Determination of Iron

A. Calibration Curve
(show calculations)

<table>
<thead>
<tr>
<th>Concentration of Fe</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg Fe/mL)</td>
<td></td>
</tr>
<tr>
<td>1. 0.0010</td>
<td>0.17</td>
</tr>
<tr>
<td>2. 0.0020</td>
<td>0.33</td>
</tr>
<tr>
<td>3. 0.0030</td>
<td>0.52</td>
</tr>
<tr>
<td>4. 0.0040</td>
<td>0.66</td>
</tr>
<tr>
<td>5. 0.0050</td>
<td>0.83</td>
</tr>
</tbody>
</table>

From $A = abc$

$$a = \frac{A}{bc}$$

$$= \frac{0.17}{(1.0 \text{ cm})(1.0 \text{ ppm})}$$

$$= 0.17 \text{ cm}^{-1}\text{ppm}^{-1}$$

Do the above data obey the Beer-Lambert law? Yes

Why? There is a linear relationship between absorbance and the concentration of Fe

---

B. Unknown Determination

1. Sample mass 0.0943 g  Volume of solution 50.0 mL

<table>
<thead>
<tr>
<th>Absorbance or %T</th>
<th>Concentration of Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg Fe/mL)</td>
<td></td>
</tr>
<tr>
<td>Trial 1</td>
<td>1.22; % T = 6.0</td>
</tr>
<tr>
<td>Trial 2</td>
<td>1.23; % T = 5.9</td>
</tr>
<tr>
<td>Trial 3</td>
<td>1.21; % T = 6.1</td>
</tr>
<tr>
<td>Mean</td>
<td>1.22; % T = 6.0</td>
</tr>
</tbody>
</table>

$$c = \frac{A}{ab}$$

$$= \frac{1.22}{(0.17 \text{ cm}^{-1}\text{ppm}^{-1})(1.0 \text{ cm})}$$

$$= 7.2 \text{ ppm}$$

2. Concentration of Fe in mg/mL 0.0072 ± 0.00006

$$\frac{0.0072 + 0.0072 + 0.0071}{3} = 0.0072$$

3. Standard deviation (show calculations)

$$SD = \sqrt{\frac{2(0.00)^2 + (0.0001)^2}{2}} = 7 \times 10^{-6}$$
4. Percent Fe in original sample (show calculations) 19.0% ± 0.2

0.0943 g diluted to 50 mL → 0.001886 g/mL

1 mL of this diluted to 50 mL → 0.0000377 g/mL or 0.0377 mg/mL

0.00718 mg/mL × 10² = 19.0% Fe

0.00377 mg/mL

0.00712

0.00377 = 19.0% Fe

0.00724 mg/mL × 10² = 19.2% Fe

s = \sqrt{\frac{(0.0)^2 + (0.2)^2 + (0.1)^2}{2}} = 0.2

\frac{19.0 + 19.2 + 18.9}{3} = 19.0

QUESTIONS

1. Why is the line on the cuvette always aligned with that of the sample holder?
   To minimize any irregularities in the glass, they will have a constant effect.

2. Why was hydroxylamine hydrochloride added to your sample?
   To reduce Fe³⁺ to Fe²⁺.

3. Iron(II) reacts with water by a hydrolysis reaction. In order to prevent this hydrolysis, acid has been added to the standard iron solution. How would your final results change if no acid has been added to the standard iron solution?
   The concentration of [Fe(C₆H₅NO₂)₃]²⁺ would be less than it should be, and therefore, the absorbance would have been less. Hence, % Fe would be low.

4. Suppose a solution of Co(NO₃)₂ has an extinction coefficient of 5.1 L/mol-cm at 505 nm. On the graph paper provided, plot a graph of A versus C (mol/L) for solutions of 0.020, 0.040, 0.060, 0.080, and 0.100 M Co(NO₃)₂ in a 1-cm cell. On the same graph, plot the percent transmittance, %T, of each solution versus concentration.

   \log %T = 2.00 - A
   A = abc
   = 5.1 \text{ L/mol-cm} \times 1 \text{ cm} \times C
   = 5.1 \text{ L/mol} \times C
   \begin{align*}
   &0.020 \quad 0.020 \quad 62.52 \\
   &0.040 \quad 0.102 \quad 79.07 \\
   &0.060 \quad 0.204 \quad 79.07 \\
   &0.080 \quad 0.306 \quad 49.43 \\
   &0.100 \quad 0.408 \quad 39.08 \\
   &0.100 \quad 0.510 \quad 30.90 \\
   \end{align*}

5. An 8.64 ppm (1 ppm = 1 mg/L) solution of FeSCN²⁺ has a transmittance of 0.295 when measured in a 1.00-cm cell at 580 nm. Calculate the extinction coefficient at this wavelength.

   A = 2.00 - \log %T = 2.00 - \log 29.5
   = 2.00 - 1.47 = 0.53

   \[a = \frac{A}{bc} = \frac{0.53}{(1.00 \text{ cm})(8.64 \times 10^{-3} \frac{g}{L}/113.9 \text{ g/mol})} \]
   \[= 7.0 \times 10³ \text{ L mol}^{-1} \text{ cm}^{-1}.\]
Calibration Curve

\[ A = abc \text{ where } ab = \text{slope} \]

\[
\text{slope} = \frac{1.00}{6.00 \text{ ppm}} = 0.167 / \text{ ppm}
\]

\[
a = \frac{\text{slope}}{b} \quad b = 1.00 \text{ cm}
\]

\[
a = \frac{0.167}{(1.00 \text{ cm})(\text{ppm})} = 0.167 / \text{ cm-ppm}
\]
Experiment 9: Determination of Iron with 1,10-Phenanthroline

PURPOSE:

This exercise reviews the fundamental concepts of quantitative spectrophotometric analysis.

THEORY:

Harris, D. C. (2003); "Quantitative Chemical Analysis 6th ed."; 258-261, 407-422, first figure @ pp. 453, 461-476, 707-709.

In this experiment, the amount of iron present in a sample is determined by first reacting the iron with 1,10-phenanthroline to form a colored complex and then measuring the amount of light absorbed by this complex. Beer's law can then be used to determine the concentration relative to absorption: 

$$A = ebc$$

To form a complex, the iron must be first reduced to its ferrous state. This reduction is done by reacting the iron with hydroxylamine hydrochloride by the following reaction:

$$2 \text{Fe}^{3+} + 2 \text{NH}_2\text{OH} + 2 \text{OH}^- \rightarrow 2 \text{Fe}^{2+} + \text{N}_2 + 4 \text{H}_2\text{O}$$

Then the reaction with 1,10-phenanthroline is:

Once a colored complex is formed, the wavelength of light which is most strongly absorbed is found by measuring the absorbance at various wavelengths between 400 - 600 nm. After the most suitable wavelength is determined, a series of iron standards is measured at this wavelength and a calibration plot of absorbance vs. concentration is prepared. The absorbance of the unknown sample is measured and the calibration curve is used to calculate the concentration of iron in the sample.

PRELAB EXERCISE (2 pts./e.a.):

Harris, D. C. (2003); "Quantitative Chemical Analysis 6th ed.": 18-8, 18-9

Also discuss the following:
1. What is a chromophore?
2. Why it is necessary to wait for at least 10 min. before reading the laboratory samples on the spectrophotometer?
3. What is a chelating agent?
EXPERIMENTAL:

NOTE: All iron solutions should be discarded into a "Heavy Metal" waste container.

1. Obtain your unknown in a 100-mL volumetric flask turned in to the TA the previous week.
   - You should provide a clean, labeled 100-mL volumetric flask to the TA for your unknown one week prior to this experiment.

2. Obtain 100 mL of stock ferrous ammonium sulfate (Fe(NH₄)₂(SO₄)₂·6H₂O) solution from the teaching assistant in a clean Erlenmeyer flask. Be certain to note the mass of ferrous ammonium sulfate used by the TA to make the solution and the final volume of the solution prepared by the TA.

3. Label five additional 100-mL volumetric flasks as Std. 1, Std. 5, Std. 10, Std. 25 and blank.

4. Into the standard volumetric flasks, pipette 1, 5, 10 and 25 mL aliquots of the standard iron solution.

5. To each of the 6 flasks, add 1 mL of ~1.4 M hydroxylamine hydrochloride, 10 mL of ~5 mM 1,10-phenanthroline, and 8 mL of ~1.2 M sodium acetate (a buffer). DO NOT PUT YOUR PIPETTES INTO THESE SOLUTIONS; pour a small amount into your beaker and pipette from this. Be sure to add the reagents in the order shown here.

6. Fill each flask to the mark with deionized water, mix well and allow to stand for 10 minutes.

7. Find a matching set of cuvettes; fill one cuvette with the analytical blank and fill the other with Std. 10.

8. Set the wavelength at 400 nm and zero the instrument.

9. Measure the absorbance of the standard in 20 nm increments in the range of 400 to 600 nm; you must re-zero the instrument every time you change the wavelength.

10. Determine the wavelength in this range which resulted in the maximum absorption. From this wavelength, measure the absorbance at +/- 15 nm in 5 nm increments. (a 30 nm range).

11. Set the wavelength to the wavelength where the maximum absorbance was observed and measure the absorbance of all standards and the unknown.

NOTE: All iron solutions should be discarded into a "Heavy Metal" waste container.

Notes:
1. For the calibration curve, select the x-y scatter chart that shows only the data points, then perform a best-fit line of your data without forcing the line through the origin.

2. To determine ppm iron, you need to know that ferrous ammonium sulfate is Fe(NH₄)₂(SO₄)₂·6H₂O and has a molecular weight of 392.14 g/mol. Note that ferrous ammonium sulfate is sometimes abbreviated as "FAS".

Outline of Calculations:
1. You need to determine the concentration of the calibration stock solution in ppm Fe (mg Fe/L). Start with the stated concentration in (g FAS / L). You can do the conversion in either of two equivalent ways:
a. Convert to (moles FAS / L), then to (moles Fe / L) then to (g Fe / L) then to (mg Fe/L) Use the fact that there is one Fe per formula unit of FAS. For other steps in this multi-step calculation you use the molar mass of FAS and the atomic mass of Fe.
b. Take (g FAS / L) and directly convert to (g Fe / L) by multiplying by the ratio of the atomic weight of Fe to the molar mass of FAS, since there is one Fe per formula weight of FAS. Then convert (g Fe / L) to (mg Fe / L)

2. Next you need to do dilution calculations for each individual iron standard solution, seeking to determine the concentration of the final solution (as tested in the spectrometer) in ppm Fe (mg Fe / L). The basic equation is conc1 x vol1 = conc2 x vol2.
   a. Conc1 would the concentration of the calibration stock solution in ppm Fe.
   b. Vol1 would be the volume of that stock solution you use. Be careful to express Vol1 and Vol2 in the same units (e.g. both in mL).
   c. Vol2 would be the final diluted volume of your diluted standard solution.
   d. Conc2 is the concentration of the diluted standard solution, in ppm Fe. Solve for this value!

3. Use the calculated concentrations of the diluted standards as the x values for graphing your calibration curve, paired with the absorbance values (as the y value) for those solutions.

4. Use the equation of your best fit line on the calibration curve to determine the concentration of iron (in ppm Fe) in your unknown sample. Use correct sig. figs. That value (the concentration of iron in the diluted solution, as measured in the spectrometer is the final value you are expected to report. You do not need to do further calculations.

Laboratory Report:

1. Name, date, and unknown number.
2. Full sample calculations, with good organization, correct units and correct sig. figs.
3. Concentration of the unknown, in ppm Fe.
4. Important chemical reactions.
5. Plot absorbance vs. wavelength (from steps 9-10) and indicate where λ_max occurs.
6. Plot absorbance vs. concentration. Be sure to include the zero concentration standard as one of your data points! Show the best fit line and its equation. Report the slope, intercept, and ε_λ_max. Also report the uncertainties in slope and intercept (sm and sb). See Harris textbook, pp. 83-84. Using Excel to calculate sm and sb is recommended (use regression analysis).
7. Submit a one page discussion of your results. It should include among others, an analysis of the standard deviations, mean results, possible sources of error and how they can be corrected.
8. Answer the discussion questions

DISCUSSION QUESTIONS:

1. Why do we use a “reagent blank” and not just distilled water to zero the spectrometer?
2. What would the effect be of waiting 30 minutes instead of 10 minutes on step 6?
3. Today's unknown samples are simple iron dissolved in water. If our sample were more complex, it might contain other compounds, some which might be colored, including some which might absorb light at the same wavelength as the iron phenanthroline complex. What problem would this cause? How could we modify our procedure to correct for this problem?
4. State the function of each reagent in this experiment: 1,10-phenanthroline, hydroxylamine hydrochloride, sodium acetate.
5. The analytical sensitivity of the method depends on the slope of the calibration curve. Was your method sensitive for the determination of iron in these samples? Why?
CH 426
Visible Spectroscopy
Determination of Iron in Lithia Water

NOTE: It is your responsibility to obtain a Lithia water sample. See the stockroom for a sample bottle.

Background

Complexation reactions are based on the reaction of a metal cation (M) and a ligand (L), which can be represented by the general equation:

\[ xM + yL \rightleftharpoons M_yL_x \]

These reactions are widely used in analytical chemistry, especially for the quantitation of metal ions. Absorption spectroscopy is a powerful tool for the determination of metals because the majority of metal-ligand complexes strongly absorb radiation in the visible portion of the electromagnetic spectrum.

The iron content of lithia water, a mineral water that originates from Lithia Springs in the Ashland area, can easily be quantified through the reaction of ferrous ion and 1,10-phenanthroline, a bidentate complexing ligand. The iron(ll)-phenanthroline complex forms easily and is quite stable. The reaction (without the actual stoichiometry) is shown below.

\[
\begin{align*}
\text{Fe}^{2+} + 3 \text{1,10-phenanthroline} & \rightarrow \text{Fe}^{3+} \left[ \text{1,10-phenanthroline} \right]^{2+} \\
\text{Iron(II)-1,10-phenanthroline complex} &
\end{align*}
\]

The reaction product of ferrous ion with 1,10-phenanthroline is shown below:

This complex absorbs strongly in the visible region of the electromagnetic spectrum where neither ferrous iron nor 1,10-phenanthroline absorbs. As with any form of absorption spectroscopy, the absorption of this complex is linearly related to its concentration via Beer's Law. The standard addition method is used to ensure there are no matrix effects from the Lithia water.
Equipment and Glassware

1. Cary 1E UV-Visible Spectrophotometer:
2. Two Quartz Cuvettes, 1.00-cm pathlength
3. pH meter with combination pH electrode and stirbar/stirplate
4. Hot plate
5. Eppendorf Adjustable Pipet (10 - 100 μL) and yellow pipet tips
6. Volumetric Pipets
   a. Two 1-mL
   b. One 4-mL
   c. One 10-mL
7. Volumetric Flasks
   a. One 10-mL
   b. Seven 25-mL
   c. One 50-mL
   d. One 250-mL
8. Beakers
   a. One 100-mL
   b. One 250-mL
9. One 250-mL Erlenmeyer flask

Reagents

1. Iron standard (1000 ppm)
2. 1,10-Phenanthroline Monohydrate
3. Glacial Acetic Acid
4. Sodium hydroxide, 50% (w/w)
5. Hydroxylamine Hydrochloride

Procedure

1. Lithia Water Digestion

   Measure 50 mL of Lithia water in a volumetric flask. Pour the water into a 250 mL Erlenmeyer flask, and rinse the volumetric flask three times with small aliquots (~2 mL) of distilled water. Pour the rinsings into the Erlenmeyer flask. Add 40 drops (~2 mL) of concentrated HCl to the digestion flask. Add 0.10 g of hydroxylamine hydrochloride to the flask and dissolve. Bring the sample to a boil and digest until the volume is reduced to 15 - 20 mL.

   During the digestion, warmup the instrument and make the rest of your solutions! You'll not have to stay beyond 3 hours if you are efficient with your time.

   After digesting the sample, remove the flask from the hotplate and allow the flask to cool. Once cooled, quantitatively transfer the sample back to the original 50 mL volumetric flask. Quantitative transfer requires several rinses (approximately 8 - 10) of the Erlenmeyer flask with small volumes of distilled water, each volume being transferred to the volumetric flask. Fill to the mark with distilled water.

2. Instrument warmup

   Follow the directions for the operation of the Cary 1E spectrophotometer. Allow 15 minutes before use so that the instrument is properly warmed up.
3. Preparation of 0.5% (w/v) 1,10-phenanthroline solution

Approximately 0.12 g of 1,10-phenanthroline monohydrate should be massed on the analytical balance. Transfer this to a 25 mL volumetric flask and fill it to the mark with distilled water. The 1,10-phenanthroline reagent is hard to dissolve so warm the solution in a beaker of hot water (the 100 mL beaker is perfect for this) until the reagent dissolves. If you wish to sonicate the solution to help dissolve the reagent, perform this step before warming the solution: sonicating the solution after warming will simply allow the reagent to crash out of solution. **Do not under any circumstances put the volumetric flask directly on the hotplate! Heating the flask in such a manner will destroy the calibration of the flask!**

4. Preparation of 10% (w/v) hydroxylamine hydrochloride solution

Approximately 1.0 g of hydroxylamine hydrochloride should be massed on the analytical balance. Transfer this to a 10 mL volumetric flask and fill to the mark with distilled water. Dissolve the reagent by shaking well.

5. Preparation of a 1.0 M acetic acid - sodium acetate buffer, pH 4.0

Add approximately 150 mL of distilled water, 15 mL of glacial acetic acid, and a clean stirbar into a 250 mL beaker on a stirplate. Using a standardized pH meter (see your instructor if there are any difficulties), place a clean pH electrode into the solution and adjust the pH to 4.0 with dropwise additions of 50% (w/w) NaOH. Pour the beaker contents into a 250 mL volumetric flask and dilute to the mark with distilled water.

6. Preparation of Solutions for Standard Addition

a. Set up six 25 mL volumetric flasks. Label them Flask #1 - Flask #6. Pipet 4.00 mL of the digested Lithia water into each of the first five flasks. Do not pipet Lithia water into Flask #6.

b. Using the Eppendorf pipet, add the following volumes of 1000 ppm iron standard:

i. Flask #1: 0 µL
ii. Flask #2: 25 µL
iii. Flask #3: 50 µL
iv. Flask #4: 75 µL
v. Flask #5: 100 µL
vi. Flask #6: 0 µL
c. Add 10.00 mL of acetic acid - sodium acetate buffer to each flask (#1-6). Swirl to mix.
d. Add 1.00 mL of the 10%(w/v) hydroxylamine hydrochloride to each flask (#1-6). Swirl to mix.
e. Add 1.00 mL of the 0.5%(w/v) 1,10-phenanthroline solution to each flask (#1-6). Swirl to mix.
f. Dilute each flask to the mark with distilled water and mix well. Wait 10 minutes for the color to fully develop. **In the meantime, clean up whatever glassware you can.**

7. Running a blank and recording the Iron(II)-1,10-Phenanthroline Spectrum

a. Flask #6 is your blank. Fill both of the quartz cuvettes with the blank solution and place them into the spectrophotometer according to the spectrophotometer instructions.
b. Make sure that Baseline Correction is selected from the Baseline Tab in the Cary Setup routine. If you have selected Baseline Correction, a Baseline button should appear under the Setup button in the main window. Click on the baseline button and record the baseline between 350 and 800 nm. After recording the baseline, right click on the window and select Clear All Traces.
c. Fill the sample quartz cuvette with the solution from Flask #1 and place it into the spectrophotometer according to the spectrophotometer instructions.
d. Obtain a spectrum of the iron(II)-1,10-phenanthroline complex according to the spectrophotometer instructions. Record the spectrum between 350 and 800 nm. Print out the report and record the absorbance at the peak maximum in your lab notebook.
e. Obtain a UV-Vis absorbance spectrum for each of your solutions, making sure that you record the peak absorbance for each solution.

Calculations

1. Using the absorbance data, create a standard addition calibration curve. Use the lecture notes and/or your text to perform the standard addition calculations. If your "concentration" is plotted as spike mass, the result of your calculations will be in units of mass. If your "concentration" is plotted as spike volume, you will have to convert your spike volume to a spike mass, which can be done by multiplying the spike volume with the spike concentration.

2. How does your result compare with the stated concentration in Appendix V (Lithia Water Analysis)? Discuss why there may be real differences between the iron concentration in your Lithia Water sample and Lithia Water samples obtained in the past. One consideration is that the previous data was obtained using Lithia Water obtained at the wellhead, whereas you probably obtained your Lithia Water sample in Lithia Park or the Plaza.

3. Discuss why an acetate buffer is necessary in this experiment.

4. Discuss why hydroxylamine hydrochloride is necessary in this experiment.

References