

Experiment 10**SPECTROPHOTOMETRIC DETERMINATION OF IRON IN DRINKING WATER**

2 lab periods

Reading:

- 1) Chapter 17, pg 393-403, *Quantitative Chemical Analysis*, 8th Edition, Daniel C. Harris (7th Edition: Chapter 18, pg 378-387).
- 2) Chapter 4, pg 83-91, *Quantitative Chemical Analysis*, 8th Edition, Daniel C. Harris (7th Edition: Chapter 4, pg 65-72)

Objective

This lab will introduce you to the concept of spectrophotometric measurements. You will analyze water samples and standards for iron using a standard spectrophotometric method. You will learn how to prepare standards, use an Ocean Optics Spectrophotometer, and make a calibration curve using Beer's Law.

Schedule and planning

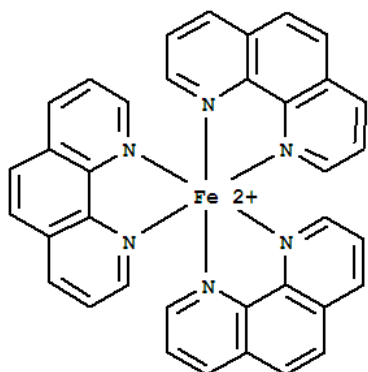
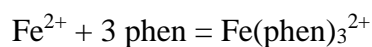
You will work in groups of *two* for this experiment. Each person must plan to bring a sample of water from home or elsewhere. You may bring more than one sample each, if you like. For example, you could bring one sample of cold water and one sample of hot water from home. Each *group* will be give one performance evaluation sample.

Lab 1 Plan ahead. Prepare your top iron standard, the hydroxylamine solution, the 1,10-phenanthroline solution, and the ammonium acetate buffer solution. You should also plan how you are going to prepare your working standards.

Lab 2 Bring in your water samples on this day. Prepare the working standards and your sample solutions for analysis. Obtain and prepare the performance evaluation sample for analysis. Perform the spectrophotometric measurements. Finish the experiment. Get help with calculations.

By and large, tap water in the United States is of good quality and safe to drink. The Environmental Protection Agency (EPA) has established standards for drinking water quality and state and local governments work hard to keep drinking water safe. The EPA standards limit the amount of iron in drinking water to less than 0.3 ppm (0.3 mg/L). Iron is not hazardous to health, but it can leave reddish-brown stains on laundry and bathroom or kitchen fixtures. In addition, iron can give the water an unpleasant taste. In this experiment, we will determine the levels of iron present in the tap water you drink to determine whether or not the water meets the established standards. You may bring bottles of tap water from home for this experiment. However, please collect the water in the morning before your class, if possible. You will also determine the iron in an unknown sample provided in the lab.

One method that is frequently used to determine trace amounts of iron in water involves using the reaction between Fe^{2+} with 1,10-phenanthroline (phen). This reaction produces a $\text{Fe}(\text{phen})_3^{2+}$ complex with a reddish-orange color which absorbs light at 510 nm. This complex is called "ferroin":



<http://www.lookchem.com/cas-147/14708-99-7.html>

Ferroin

Iron present in water exists predominantly in the form of Fe^{3+} . Since 1,10-phenanthroline reacts only with Fe^{2+} , it is necessary to reduce Fe^{3+} to Fe^{2+} . You will add the *reducing agent*, hydroxylamine, to your water sample and standards in order to reduce Fe^{3+} to Fe^{2+} . Because dissolved oxygen in water can reoxidize Fe^{2+} to Fe^{3+} , an excess amount of the hydroxylamine reducing agent is needed to keep iron in the +2 state during the course of the experiment. Fe^{2+} is quantitatively complexed by 1,10-phenanthroline in the pH range from 3 to 9. An acetate buffer is used as a buffer to maintain a constant pH at 3.5. Fe^{2+} can be oxidized to Fe^{3+} if the pH is >9 . If the pH is <3 , H^+ competes with Fe^{2+} for the basic 1,10-phenanthroline to form phenH^+ . The determination of the colored $\text{Fe}(\text{phen})_3^{2+}$ complex is performed with a spectrophotometer at a fixed wavelength of 510 nm. You will make a calibration curve based on iron standard solutions.

Prelaboratory Assignment

- 1) The iron concentration in a water sample taken from a site of suspected groundwater contamination is determined by spectrophotometry. The following results are obtained:

Concentration (M)	Absorbance Trial 1	Absorbance Trial 2	Absorbance Trial 3
0	0.010	0.011	0.009
5.0×10^{-5}	0.041	0.045	0.036
10.0×10^{-5}	0.078	0.083	0.085
15.0×10^{-5}	0.125	0.117	0.114
20.0×10^{-5}	0.161	0.163	0.170
Well water sample	0.093	0.094	0.089

Correct each measured absorbance value for the blank absorbance. Calculate the average (corrected) absorbance and the standard deviation for each set of measurements. Using Excel, or another plotting program, plot the average (corrected) absorbance vs. the concentration. Add error bars to your plot. Error bars simply indicate the uncertainty in a reported measurement.

Your error bars will indicate plus/minus one standard deviation of uncertainty. Show the equation of the calibration line and the R^2 value on the plot. Give your plot a title and make sure the axes are labeled appropriately and have units. Calculate the concentration of iron in the well water sample from the equation of the calibration line determined using Excel. Use equation 4-27 in your text to determine the uncertainty in the iron concentration in the well water (hint: read pgs 83-91 of your text. These calculations are simple when Excel is used. Section 4-9 of your text has a useful description of how to use Excel for this purpose (pg 89-91).)

Apparatus

- Ocean Optics Spectrophotometer and LabQuest
- 2 plastic cuvettes: make sure they are not scratched or damaged
- Plastic bottles (or equivalent): 30 mL (1), 125 mL (1), 250 mL (1), 60 mL (2)
- 50-mL volumetric flasks
- 100 mL volumetric flasks
- Volumetric pipets: 1, 5, 10, 25 mL
- 10-mL graduated cylinder
- Measuring pipets; 1, 5 mL
- Beakers, small bottles or test tubes: ~25 for each group (may be plastic)
- Parafilm or plastic wrap

Chemicals

- Hydroxylamine hydrochloride
- 1,10-phenanthroline monohydrate
- Ammonium acetate
- Glacial acetic acid
- Concentrated HCl
- 1000 mg/L iron standard solution

Procedure

- 1. Prepare reagent solutions**—these do not have to be prepared using volumetric glassware or the analytical balance! These solutions are stable for several months. You should store them in plastic bottles you can check out from lab services.
 - a) Hydroxylamine hydrochloride solution: You will need a total of 20-30 mL. Dissolve 3 g of hydroxylamine hydrochloride in 30 mL DI water.
 - b) Ammonium acetate buffer solution: You will need a total of 100-200 mL of this. Dissolve 55 g ammonium acetate in 45 mL water. **Carefully** add 150 mL glacial acetic acid. Do this in the hood! Mix.
 - c) Phenanthroline solution: Dissolve 100 mg 1,10-phenanthroline monohydrate in 100 mL water. Add two drops conc. HCl to your solution. Stir to dissolve.
- 2. Preparing your iron top standards**—these *do* need to be prepared using volumetric glassware.
 - a) Pour a small amount of the 1000 mg/L iron standard solution into a small beaker. Use the 1000 mg/L iron standard solution to prepare 50-mL of 200 mg/L iron solution. Use the 200 mg/L standard to prepare a 50-mL standard that is 20 mg/L in iron. These two standards will be your “top” standards. You will use these to prepare all your other standards. Once you have prepared these standard solutions, pour them into 60 ml bottles and store them until you are ready to prepare your “working” standards.
- 3. Preparing your “working” standards and blank--** this should be done on the same day

you will do the spectrophotometric analyses. Allow a minimum of 10 minutes for color development in 50 or 100-mL beaker.

- a) For the blank: add ~30 mL DI water to a 50 mL volumetric flask. To this, add 1 mL of the hydroxylamine hydrochloride solution and mix. Then add 10 mL of the buffer solution and 4 mL of the phenanthroline solution. Mix and then dilute to the mark with DI water. Mix thoroughly. Note: you can use a 10 mL graduated cylinder to add the buffer solution, and measuring pipets to add the other reagents. Pour the mixed solution from the volumetric flask to a small beaker or other container (i.e. test tube). You need < 5 mL of this solution to do the spectrophotometric analysis. Repeat the procedure two more times. You should have three samples of the blank (replicates) in three separate beakers when you have finished. Cover each beaker or container with parafilm or plastic wrap. Label.
- b) Use your top standards (from Step 2, the 20 and/or 200 mg/mL standards) to prepare at least four working standards that range in concentration from *about* 1.0 mg/L to 10.0 mg/L. To do this, calculate the volume of a particular top standard needed to prepare a chosen working standard. Add this volume to 10 mL of DI water in a 50 mL volumetric flask and add the reagents as in a) above (hydroxylamine hydrochloride, buffer and phenanthroline) and dilute to the mark. Choose concentrations that are easy for you to prepare using available volumetric glassware. It is strongly suggested that you make the most dilute standard first then move up to the next highest concentration. (*Why?*) You should have three separate replicates for each standard.

If you reuse your volumetric flasks or pipets, rinse them out between samples. Always prepare solutions starting with the lowest concentration first. Keep your solutions covered to minimize their exposure to air.

4. Preparing your tap water samples and performance evaluation sample

- a) Tap water samples: Use your volumetric pipets to add 30 mL of your tap water sample to a very thoroughly rinsed 50 mL volumetric flask. Add the reagent solutions, as above. Bring to a total volume of 50 mL using DI water. Wait for color development. If the color of your solution is darker than that of the darkest standard, prepare again, using less water sample. Note how much water sample you have used. You will need this in your calculations. Prepare three replicates.
- b) Performance evaluation sample: Use your volumetric pipets to add 10 mL of your check standard to a very thoroughly rinsed 50 mL volumetric flask. Add the reagent solutions, as above. Bring to a total volume of 50 mL using DI water. You will need this in your calculations. Prepare three replicates.

5. Spectrophotometric measurements—you will use LabQuest with an Ocean Optics Spectrophotometer to measure the absorbance values of your standards and samples. Always start your measurements with the blank, and then analyze your standards in order from lowest to highest. Analyze your tap water samples and performance evaluation sample last. Make sure you rinse your cuvette well between samples/standards. The cuvettes you will use have a pathlength (b) of 1 cm.

Instructions for using LabQuest with the Ocean Optics Spectrophotometer.

Note: use the stylus, not your finger to touch the LabQuest screen. Make sure you place the cuvette in the spectrophotometer so that the light shines through the clear portion of the cuvette! Use the same cuvette for all your measurements.

- 1) Place a cuvette containing DI water in the cell compartment of your ocean optics instrument and push “sensors”, go to ” calibrate” and then “USB spectrometer”. Wait for the lamp to warm up. Push “Finish calibration”, then “ok” on LabQuest. This is not a true calibration, but merely zeros the spectrometer using DI water.
- 2) Discard the DI water and rinse the cuvette with one of your standards. Add ~1 mL of the standard to the cuvette and place in the cell compartment of your Ocean Optics spectrometer. Click “play” on your LabQuest.
- 3) Push “stop”. The LabQuest registers the wavelength associated with the highest absorbance value. Double check to ensure that the wavelength registered on your LabQuest is the same, or very close, to as the wavelength at which the colored $\text{Fe}(\text{phen})_3^{2+}$ complex absorbs (510 nm).
- 4) Measure the absorbances of your blank, standards and samples at 510 nm.

References

Standard Methods for the Examination of Water and Wastewater: Including Bottom Sediments and Sludges. 1998. Published by the American Public Health Association, American Water Works Association, and the Water Pollution Control Federation.

Report

A typed report is required for this experiment (100 points). Each partner must turn in his/her own report. A professional style of writing is expected. *Make sure to use your own words! Plagiarists will be given a 0 on this report.*

Your report should contain:

- 1) Title page, with the title of the experiment, the date, the lab section, and the names of your group members.
- 2) A short introduction which includes the experimental and educational objectives of the experiment, a description of your samples, the measurements you made and the technique you used.
- 3) A brief summary of the experimental procedures used.
- 4) Results: you should consider/complete the following:
 - a) Prepare a table similar to that in the prelab assignment. In your table, provide your raw data for each trial, and the average, and the standard deviation for each set of trials. Also, calculate the average *corrected* absorbance and the standard deviation for each set of measurements.
 - b) Using Excel, or another plotting program, create a standard curve by plotting the average absorbance vs. the concentration. Add error bars to your plot. Show the equation of the calibration line and the R^2 value on the plot. Give your plot a title and make sure the axes are labeled appropriately and have units. Does your plot contain a nonlinear region? What should you do if it does? Determine the R^2 value for the linear region only and include this on your plot as well. Because you used corrected absorbances to make your plot, the intercept should, in principle, be zero or very close to it.
 - c) Calculate the concentration of iron in the tap water samples and the performance evaluation sample from the equation of the calibration line determined using Excel. *Do not forget to consider the dilution factor in your calculations!* Use the method of least squares to calculate the uncertainty in the slope, the intercept and to determine the uncertainty in the iron concentration in the tap water samples and the performance evaluation sample.
 - d) Calculate the molar absorptivity of $\text{Fe}(\text{phen})_3^{2+}$ at 510 nm. Compare your value with the literature value, $11,100 \text{ M}^{-1}\text{cm}^{-1}$. *Watch your units!* Explain the cause for the difference, if any is observed.
- 5) Discussion
 - a) Discuss, interpret and comment on your results. Do the water samples meet the Federal standards for drinking water? Are there any significant differences in iron concentration observed between the water samples your group analyzed? Discuss potential reasons for differences/similarity in iron concentration. Comment on the accuracy and precision of your results. Do you have confidence in your data? Why or why not?

If the concentration of iron in the tap water is very low, the absorbance will be very small (e.g. <0.001) and the concentration may not be determined accurately. Be sure to mention the two common approaches (that will increase the absorbance) to increase the accuracy of the measurement. (Hint: think about Beer's law: $A = \epsilon bc$) Note: if the concentration is below the established limit of detection (LOD) most analytical labs

would report iron as “nd” (not detected or detectable) unless you explicitly told them to try and get a measurement.

- b) Discuss any problems you encountered in this experiment and how you addressed these problems. How would you change the experiment to improve the quality of the data?
 - c) Discuss advantages and disadvantages of the technique you used in terms of the analysis of iron concentration in water. What other methods could be used?
- 6) Conclusions. What conclusions can you draw from your data? Is spectrophotometry an appropriate tool for the measurement of iron in water? Why or why not?