Chemistry 141 Laboratory
Spectrometric Determination of Iron Concentration
Lab Lecture Notes
8/29/2011
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What is the purpose of this experiment?

We're going to learn a way to quantify the amount of material in an unknown sample. This technique is called spectrometry.

What is Spectrometry?

Spectrometry is a technique that uses the amount of light absorbed by a substance to determine its concentration. In this experiment we will be using the amount of visible light absorbed by an iron complex as a way of determining the amount of iron in an unknown.

What do we actually measure in spectrometry?

We measure two characteristics of the light that's absorbed. One is called the wavelength of the light absorbed. The wavelength of the light is the distance in nanometers (10^{-9} meters) between the peaks of the light waves.

The second is called the intensity of the light absorbed. The intensity of the light absorbed is simply how much of the light is absorbed.

What is visible light?

Visible light is that portion of the electromagnetic spectrum that we can see. Other portions include the infrared (longer wavelengths than red light), and ultraviolet (shorter wavelengths than violet light), which are invisible. Visible light has wavelengths between about 650 nm and about 420 nm. Visible light when passed through a prism or bounced from a grating can be broken down into its component colors, the colors of the rainbow. The order of these colors from longest to shortest wavelength is given by the mnemonic device

ROY G BIV

for Red Orange Yellow Green Blue Indigo Violet. One of your tasks today will be to assign wavelength ranges for each of these visible colors. (You can skip the indigo, since it's so hard to tell from plain old blue.)

How are frequency and intensity measured?

With a device called a spectrophotometer. A spectrophotometer has the following components:

> A light source.
> A light dispersion device (a prism or grating)
> A pair of slits (to select among the dispersed wavelengths)
> A sample cell
> A detector of some sort.

Here's a crude schematic of a spectrophotometer:
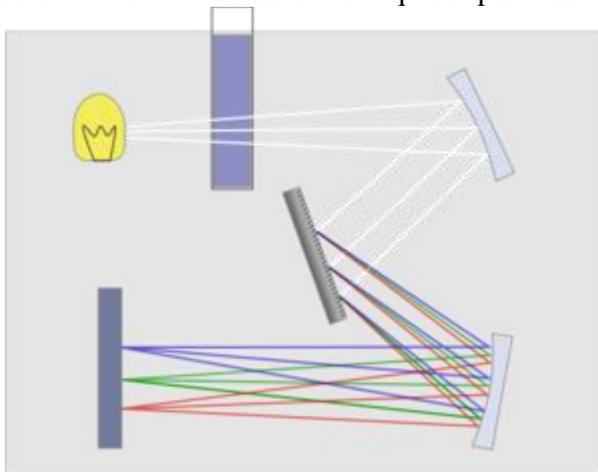
How does a spectrometer work?

It works by comparing the amount of light detected when the sample is there (I) with the amount of light detected when the sample is not there (I^0). A small computer within the spectrometer then converts this to either Transmittance (%T) or absorbance (A).

%T is defined as $\%T = \dfrac{I}{I^0} x100\%$

A is defined as $A = \log\left(\dfrac{I^0}{I}\right)$.

How does this help us find the concentration of our unknown?

Concentration is related to absorbance by the Beer-Lambert law:

$$A = \varepsilon bc$$

In this equation, c is the concentration of the substance in question, b is the path length of the light through the sample, usually set to one cm, and ε is a number called the extinction coefficient, which depends on wavelength, and says how strongly a molecule absorbs at that wavelength.

Therefore at a given wavelength, the higher the absorbance, the higher the concentration.

How do we decide what wavelength to use?

We take a spectrum, and choose the wavelength where the absorbance is highest, called λ_{max}.

What is a spectrum?

A spectrum is a graph of wavelength vs absorbance or wavelength vs transmittance. We will measure both absorbance and transmittance for all parts of the experiment.

Why do we choose λ$_{max}$ as our wavelength?

The absorbance will be largest at λ_{max}. When measuring the absorbance of a sample, the larger the absorbance is, the more precise the measurement is. So measuring our absorbance at λ_{max} maximizes the precision of our measurement.

How do we use the Beer-Lambert law to figure out our concentration?

We can solve the Beer-Lambert law for concentration. This yields:

$$c = \frac{A}{\varepsilon b}$$

A we measure, and you said b equals 1 cm, but what do we do if we don't know the extinction coefficient ε?

We create a calibration curve, by measuring the absorbance of several solutions of known concentration, and then plotting them on a graph, with the absorbance (A) on the y axis, and the concentration in units of mg/mL on the x axis. We then measure the absorbance of our unknown, and by comparing to our standard curve, determine the concentration.

How do we determine the concentration by comparing our absorption to the standardization curve?

Since we expect our calibration curve to make a straight line, we can use regression analysis to determine the equation of the straight line, just as we did in our excel practice in the first week. If we instruct excel to display the equation, it will be in the form

$$y = mx + b$$

In this equation y will be our absorbance and x will be our concentration. If you substitute your measured absorbance for y, then you can solve for the concentration of your unknown.

What if our unknown is colorless (has no visible absorption)?

In that case, we mix it with a substance that will react with it in such a way that the product is both soluble and colored. Such a substance is called a complex.

Do we need to do this?

In this experiment, we will use the complex formed between Fe^{+2} and an organic molecule called ortho-phenanthroline, $C_{12}H_8N_2$, to make it easier to detect the presence of iron. The reaction between them is:

$$3\,C_{12}H_8N_2(aq) + Fe^{+2} \rightarrow [(C_{12}H_8N_2)_3\,Fe]^{+2}$$
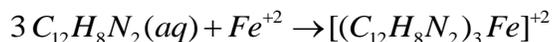
The resulting solution has a red-orange color.

The reaction you just wrote is for Fe^{+2} only, but iron can be either Fe^{+2} or Fe^{+3}, and we're supposed to measure the total iron. How do we deal with this problem?

The problem is resolved by putting a solution in your reaction mixture that ensures that all the iron is reduced to Fe^{+2}. In this case we use hydroxylamine hydrochloride. The balanced reaction is

$$4Fe^{+3} + 2NH_2OH + 3H_2O \rightarrow 4Fe^{+2} + N_2O + 4H_3O^+$$
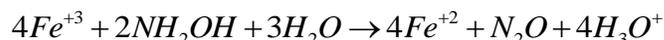
Is the concentration of the ferroin solution we study the same as that of our unknown?

No! In making the ferroin solution, your actually taking 1 mL of your unknown, and adding enough other solutions to reach a final volume of 50 mL.

Well, how do we find the concentration of our unknown?

We use an equation called the lever rule,

$$M_i V_i = M_f V_f$$

In this equation, M_i and M_f are the initial and final concentrations in units of moles per liter, and V_i and V_f are the intial and final volumes of the solution. In this case, we know the final concentration, the final volume (50.00mL) and the initial volume (1.000 mL), so our unknown will be the initial concentration.

Wait! In the lever rule, the concentration is in moles/L, but our concentrations are in mg/mL. Doesn't that matter?

No. The reason is that in both the initial and final concentrations we're dealing with mg of iron. Since the initial and final concentrations use the same units, the lever rule works.

Experimental Tips

- Make your solutions for parts C and D first. This will allow them to develop for the 20 necessary minutes while you're doing other parts of the experiment.

- Measure your solutions directly into your volumetric flasks.

- While the color is developing do the chalk experiment. DO NOT THROW THE CHALK AWAY.

- When assigning colors to wavelengths in the chalk experiment, don't freak out over whether a color is exactly blue or exactly green, or exactly orange or exactly red. We understand that color descriptions near the boundary between two colors will be extremely subjective. As long as the wavelength ranges you obtain in this part are reasonable, you'll be fine.

- After the chalk experiment, determine λ_{max} for one of your standard solutions. It is not necessary to wait the full 35 minutes before doing this. To form your spectrum, take measurements of absorbance and % transmittance every 20 nm beginning with 400 nm and going to 600 nm. Then refine your result by choosing the 20 nm interval in which the absorbance is highest, and collecting the absorbance every 5 nm for that interval.

- Collect data for your standard curve after setting your wavelength to λ_{max}

- The concentration of the standard iron solution you'll be using to create your calibration curve is 5.00×10^{-2} mg/mL

- Remember that each person has their own individual unknown. Thus each group will perform the spectroscopic measurement on one unknown for each group member.

- Remember that each time you change the wavelength you need to reinsert your water blank, and rezero the instrument.

Lab Report

For your lab report you need to turn in the following:

- Carbon copies of your lab notebook pages (today)

- Your prelab (today)

- A color line for your data in part B (described on p. 11)

- A plot of absorbance vs. wavelength for the data in part C. From this determine λ_{max} and enter it in on your report sheet.

- A calibration curve for your standard solutions. Include the equation for the line and your R^2 value.

- A determination of the ferroin concentration in your unknown. Calculate your average absorbance, and use this average with your calibration curve to determine the concentration of the ferroin. Record this on your Data Report sheet.

- Use the concentration above, along with the initial volume if the unknown iron solution you pipetted into the volumetric flask to determine the initial concentration of iron in your unknown.

- Sample calculations showing how you determined the concentration of Fe in your standards (choose one of the five standards) and your unknown.

- Brief comments on the accuracy of the data you used in determining your calibration curve and any sources of error you encountered in the experiment. Also comment on whether your data in D are consistent with Beer's law. Why or why not?

Honor Stuff

All experimental work will be collaborative. You can do all work involving your standard solutions together, including the plotting and regression analysis on the calibration curve. In addition, you may generate your color lines collaboratively with your lab partner.

Each of you will have a different unknown, so you'll have to do the calculation of the ferroin concentration in your unknown, and the original iron concentration in your unknown, individually. The discussion questions in section viii on page 45 also need to be answered individually.