Skill Building Activity 2

Determining the Concentration of a Species using a Vernier Spectrometer

Purpose

To use spectroscopy to prepare a Beer's Law plot of known dilutions of copper(II) sulfate so that the concentration of a saturated sample of aqueous copper(II) sulfate can be determined.

Background

Spectroscopy is one of the most powerful analytical techniques in modern science. Before the advent of spectrophotometric techniques, a chemist interested in determining the amount of a particular substance present in a sample had to analyze the sample via a series of chemical reactions specific to that species and then carefully weigh the products (entire tomes exist detailing such analytical reactions and you will get to do some of these tests later in the semester). This process, however, is extremely time consuming, prone to error, and generally impractical for measuring trace amounts. Today, most routine assaying is done quickly and efficiently by means of spectroscopy.

Spectroscopy works by relating the concentration of a species in solution to the amount of light it absorbs. In this experiment we will determine the quantity of copper(II) sulfate using visible absorption spectroscopy. Because the wavelengths of light we will use are in the visible portion of the electromagnetic spectrum, our solutions will all be colored. However, this technique can also be used in other regions of the spectrum where the wavelength is not visible to our eyes, but can be measured using a photo-detector.

In a solution containing a colored compound, the intensity of the color can be used to measure the concentration of the compound; the more intense the color, the higher the concentration. The spectrophotometer measures how much light is absorbed at a given wavelength.

How a spectrometer works

White light produced by the light source is transmitted off a diffraction grating through an aperture which narrows the beam before going through the sample. Adjusting the angle of the diffraction grating allows one to select the wavelength of the incident light which strikes with the sample. If light is absorbed by the sample, the intensity of the transmitted light exiting the sample (I) will be less than the intensity of the incident light (I_o). The ratio of transmitted light intensity to the incident light intensity is the transmittance, T:

$$T = \frac{I}{I_0} \quad \text{Equation 1}$$

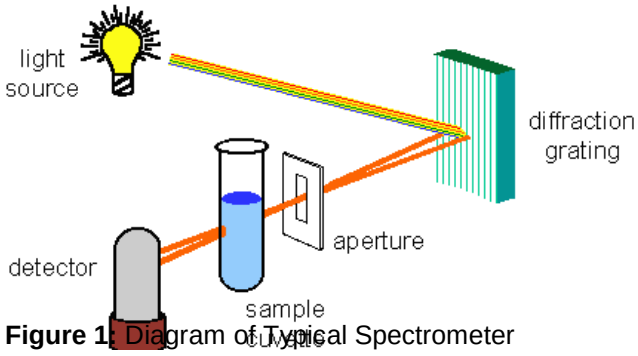


Figure 1: Diagram of Typical Spectrometer

The extent to which the light is absorbed by the sample is due to the concentration of the sample, C. When the concentration is high, many absorbing molecules will be in the path of the light and will absorb it, and less light will be transmitted. When the concentration is low, fewer absorbing molecules will be struck by the light and little light will be absorbed; thus more light is transmitted in this case. Another factor that affects the intensity of the light exiting the sample is the path length, l, which is the distance the light travels through the sample. The greater the distance the light must travel through the solution (path length), the greater the number of absorbing molecules the light will strike. The relationship between transmittance, and those factors that directly affect the intensity, l and C, is expressed in the Beer-Lambert Law (or simply Beer's Law):

$$-\log_{10} T = A = \varepsilon l C \quad \text{Equation 2}$$

where ε is a proportionality constant called the molar extinction coefficient (also known as the absorptivity) and A is the **absorbance** of light by the sample. The molar extinction coefficient

depends on the structure of the species absorbing the light and the wavelength of the light absorbed. A larger extinction coefficient means that substance is better at absorbing light. Each pure substance has its own unique extinction coefficient.

Experimental Considerations

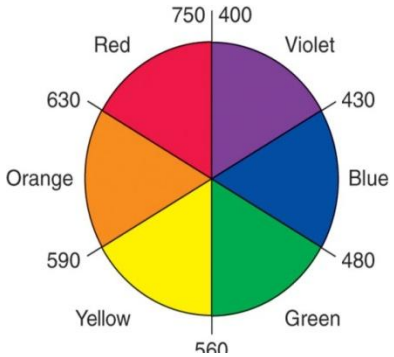
Two important questions to answer before using a spectrophotometer are: a) "What wavelength will I use to measure the absorption of my compounds?" and, b) "How will I calibrate my instrument to get accurate readings?"

a) Choosing a wavelength

The color of a substance depends on which wavelength of light it absorbs. If a substance absorbs red light only, it will appear green in color, green being the average color of all the unabsorbed wavelengths. The artist's wheel shown below becomes useful because the color a substance appears to be is directly across the wheel from the color of light that substances has absorbed.

Many substances can be identified by their colors. For example, Co^{2+} (aq) appears red because it absorbs primarily green light. On the other hand, Ni^{2+} (aq) appears green indicating that a solution of Ni^{2+} (aq) absorbs primarily red light.

Figure 2 – A color or artist's wheel



Thus to answer the question, *"What wavelength will I use to measure the absorption of my compounds?"* it is important to choose a wavelength where the solution strongly absorbs light. For example when studying a red solution it would be far better to use green light (red's complement), which is strongly absorbed by the solution; rather than orange or red light because these colors would be less strongly absorbed. The stronger the absorption at a particular wavelength the more sensitive the instrument will be at that wavelength and the more accurate your results. Thus, for a red-colored sample a wavelength in the green would probably be best; conversely if a sample is blue, then a wavelength in the orange region would probably be best.

In practice one selects one "best" wavelength for the experiment by measuring the absorption of the solution in question across this range of wavelengths and choosing the wavelength that gives the greatest absorption. For example, for the red solution described above, the experimenter might choose to measure the absorption of the sample at a range of wavelengths between 480nm and 560nm, and then narrow-in on the wavelength that gives the greatest numeric value of the absorbance.

b) Calibrating the spectrometer

Once the specific wavelength that will be used for the experiment is chosen, the experimenter needs to calibrate his or her spectrometer so that the absorbance readings can be converted into useful data such as molarity (or another unit of concentration) of the sample.

As noted on the previous page, Beer's Law relates absorbance to concentration (molarity) of a sample.

$$A = \varepsilon l C \qquad \text{Equation 3}$$

where A is the absorbance of the sample measured by the spectrophotometer, ε is the molar absorptivity (or the molar extinction coefficient), l is the path length or distance the light travels through the sample, and C is the concentration of the solution. In most instruments, the path length, l, is a constant (1.0 cm in our experiment) and can therefore be factored into ε.

Thus the Beer's Law equation can be simplified as:

$$A = \varepsilon C$$

$$y = mx$$

which looks like an equation of a line with intercept of zero. The molar absorptivity, ε, can therefore be determined by finding the slope of a plot of the absorbance as a function of concentration for a series of standard solutions of known concentrations. This is known as a *calibration curve*.

Once ε has been determined from the calibration curve, the Beer Law's equation can be used to determine the concentration of an unknown solution by measuring its absorbance under the same conditions.

Preparing your known standards and unknown solution for analysis

You will be preparing five copper(II) sulfate solutions of known concentration (standard solutions) by diluting known volumes of a 0.200 M $CuSO_4(aq)$ stock solution with deionized water. Copper(II) sulfate has an easily-identifiable vivid, bright blue color whose intensity is dependent on the solution's concentration and can be measured using spectroscopy.

Procedure

Safety:

GENERAL SAFETY: Students must wear safety goggles at all times.

WASTE DISPOSAL: All solutions used in this lab must be disposed in a waste container.

Materials and Equipment

You will need the following equipment:
8 small test tubes
two cuvettes for the spectrophotometer
one 1.00 mL volumetric pipette
one 2.00 mL volumetric pipette
one 3.00 mL volumetric pipette
one 4.00 mL volumetric pipette

Your instructor will provide you with the spectrophotometer, cuvettes, stock solution, volumetric flasks, pipettes, and unknowns. All other equipment is in your lab drawer.

1. Measure out approximately 20 mL of stock 0.200 M $CuSO_4$(aq) in a small beaker.

2. Rinse your pipette with deionized water followed by the stock solution.

3. Label six clean, dry, small test tubes 1, 2, 3, 4, 5, and 6. Fill Tube #1 approximately half-full with deionized water. Fill Tube #6 approximately half-full with stock solution. For Tubes #2-5, pipette the following volumes of stock solution and deionized water into each and mix well. *Be sure to rinse the pipettes with deionized water and standard solution first.* Calculate the concentration in molarity for Tubes #2-5.

Tube	mL of 0.200 M $CuSO_4$ used	mL of DI H_2O added	Concentration (M)
1	0	5.00	
2	1.00	4.00	
3	2.00	3.00	
4	3.00	2.00	
5	4.00	1.00	
6	5.00	0	

4. Zero the instrument with Tube #1.
5. Use Tube #6 (stock solution) to find your wavelength to measure at by the directions for the Vernier Spectrometer– a poor choice of wavelengths will result in poor data. See the introduction to this experiment for help in choosing a wavelength. If time is limited, your instructor may provide you with a specific wavelength to use.
6. Measure the absorbance of Tubes #2-6 at the wavelength found in Step 4 by filling the provided cuvettes ¾ of the way full with the solution and inserting in the Vernier Spectrometer and hitting "Keep" to record the molarity and absorbance. *Be sure to clean the cuvettes with deionized water and dry them between each use*. You will have six measured data points including the deionized water blank (which should have a zero absorbance if you properly zeroed the spectrometer).
7. Make a calibration curve using Excel by plotting the concentration of the six solutions on the x-axis and the absorbance of each on the y-axis. Your intercept should be at the origin (0,0) since your blank solution counts as one of the points. Calculate a trendline and report the equation and the R^2 value on your graph to include with your post-lab.

Analyzing your saturated solution

8. Add 1.8 g of copper(II) sulfate pentahydrate crystals to 3 mL of deionized water to a clean, dry

small test tube. While it is not important to know the exact amount of solid and liquid, it is imperative that some solid remains undissolved in the beaker at all times. If at any point all the solid does dissolve, add increments of 0.2 g more until solid reappears.

9. Cool the beaker in an ice bath while mixing thoroughly. Record this temperature (it should be close to 0°C).

10. Allow the solid to settle to the bottom of the solution. Use a plastic dropper to carefully remove solution only to another clean, dry test tube, leaving the solid behind. Using a 1.00 mL volumetric pipette, carefully pipette solution out and into a new clean, dry small test tube labeled 7. Ensure that you do not pull up any solid into the pipette. *Be sure to rinse the pipette with deionized water and stock solution first.* Pipette accurately 4.00 mL of deionized water to the last test tube that contains the $CuSO_4(aq)$.

11. Using the same wavelength that you used to construct your standard curve in Step 5, measure the absorbance of this diluted solution. *This should not be included on your graph*, only recorded. You can now use the equation of your best-fit-line to determine the concentration of the dilution of saturated $CuSO_4(aq)$.

12. Because this is a diluted solution, you will now need to calculate backwards from this concentration to determine the actual concentration of copper(II) sulfate in your original unknown sample, in molarity.

13. Report your concentration of saturated copper(II) sulfate at your recorded temperature and compare to the CRC value at 0°C, in grams per 100 mL of water.

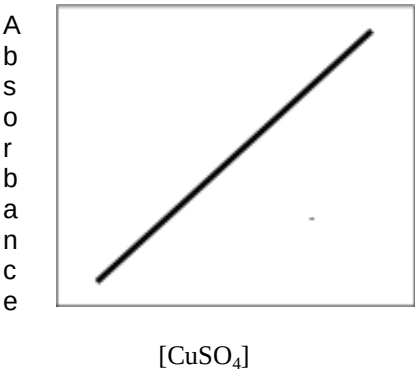


Figure 1: Beer's Law Calibration Plot

Vernier SpectroVis Spectrophotometer

(Order Code: SVIS)

SpectroVis is a portable, visible light spectrophotometer.

What is Included with the SpectroVis?

- One SpectroVis unit
- 15 plastic cuvettes and lids
- One standard USB cable
- User's Guide (this document)

Software Requirements

Logger *Pro* 3 (version 3.6 or newer) software is required if you are using a computer. The LabQuest application version 1.1 or newer is required if you are using a LabQuest. Visit the downloads section of www.vernier.com to update your software.

Using SpectroVis with a Computer

1. Install Logger *Pro* 3 software (version 3.6 or newer) on your computer before using SpectroVis.
2. Connect the SpectroVis to a powered USB port or a powered hub.
3. The first time you connect a SpectroVis, your computer may ask you a few questions. **Note:** Do not go online for device drivers. The device drivers were installed when you installed Logger *Pro* 3.

Calibrate SpectroVis

1. To calibrate the SpectroVis, choose Calibrate ► Spectrometer from the Experiment menu.
2. Fill a cuvette about ¾ full with distilled water and place it in the cuvette holder.
3. Follow the instructions in the dialog box to complete the calibration, and then click OK.

Collect Data

There are three general types of data collection measuring absorbance – absorbance *vs.* wavelength, which produces a spectrum, absorbance *vs.* concentration for Beer's law experiments, and absorbance *vs.* time for kinetics experiments.

Measure the Absorbance Spectrum of an Aqueous Sample (Absorbance vs. Wavelength)

1. Fill a cuvette about ¾ full of the solution to be tested. Place the sample in the cuvette holder of the SpectroVis.

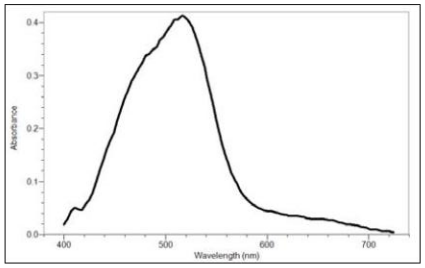


Figure 1: Typical absorbance spectrum

2. Click ▶ Collect. Click ■ Stop to end data collection.

Conduct a Beer's Law Experiment (Absorbance *vs.* Concentration)

1. Measure an absorbance spectrum as described above.
2. Click on the Configure Spectrometer Data Collection button, .
3. Select Abs *vs.* Concentration as the collection mode. The wavelength of the maximum absorbance will be automatically selected. Click OK to continue or click Clear and select a wavelength on the graph or in the list of wavelengths.
4. Place your first Beer's law standard solution in the cuvette slot. Click ▶ Collect and then click Keep. Enter the concentration of the sample and click OK.
5. Place your second standard sample in the SpectroVis. After the absorbance readings stabilize, click Keep. Enter the concentration of the second sample and click OK.
6. Repeat Step 5 for the remaining standard samples. After you have tested the final standard, click ■ Stop to end the data collection.
7. Click linear fit, , to see the best fit line equation for the standard solutions.
8. Place an unknown sample of solution in the cuvette holder. Choose Interpolation Calculator from the Analyze menu. A helper box will appear, displaying the absorbance and concentration of the unknown. Click OK.

Lab Report for Measuring Copper(II) Sulfate Using Spectrophotometry

Concentration of $CuSO_4$(aq) stock solution from bottle: _____ M

Wavelength chosen for analysis: _____ nm

Justification for choice of wavelength:

Calibration Standards Data Table:

Solution	Concentration (M)	Absorbance	Visual Appearance
Cuvette (1) (pure water)			
Cuvette (2)			
Cuvette (3)			
Cuvette (4)			
Cuvette (5)			
Cuvette (6) (pure standard)			

Show calculations for the concentrations of cuvettes (2-5) in your notebook. You will need to use $M_1V_1=M_2V_2$

Make a calibration curve by plotting concentration in molarity on the x-axis versus absorbance on the y-axis and **attach it to your report**. Being certain it goes through the origin because zeroing your instrument ensured that at zero concentration the absorbance was zero

Saturated Solution Analysis:

Temperature of ice bath: _____ °C

Volume of saturated solution used _____ mL

Volume of deionized water added _____ mL

Observed color of saturated solution: _____

Wavelength used to analyze saturated solution: _____ nm

Absorbance of saturated solution: _____

Concentration of $CuSO_4 \cdot 5\ H_2O$ in diluted saturated solution: _____ M
show calculations (hint: this is where you use the slope of your best-fit-line):

Concentration of $CuSO_4 \cdot 5\ H_2O$(aq) in original undiluted saturated sample: _____ M
show calculations:

Concentration of $CuSO_4 \cdot 5\ H_2O$(aq) in g/100 mL water: _____
g/100 mL
show calculations:

CRC value for solubility of $CuSO_4 \cdot 5\ H_2O$(aq) in 100 mL of water at 0°C _____