Name____________________ Section________________

Partner(s)__________________ Date__________________

MORE LIGHTS, COLOR, ABSORPTION!

PRE-LAB QUERIES

1. The terms absorption and transmittance are often used when describing the interaction of light with matter. Explain what each term means and how absorption and transmittance of light are related.

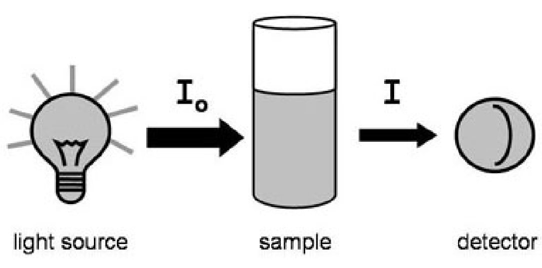
2. What possible factors influence the amount of light absorbed by a solution and how might they affect it?

OBJECT

In this activity you will explore the variables that influence the absorbance of light by a solution. From the data collected you will derive Beer's Law.

INTRODUCTION

The spectrophotometer measures the ratio of the intensity of the outgoing light (I) to the intensity of the incoming light (I_o). The light impinging on the sample in a spectrophotometer is monochromatic (one specific wavelength, λ).



The spectrophotometer can display output as **absorbance**, which is (-log I/I_o), and as **percent transmittance** (%T), which is (I/I_o x 100). Absorbance is NOT the same as **absorption**, which is (1 – T). We will quantitatively explore three variables that influence the absorbance: wavelength; pathlength; and, concentration. Some conditions that influence absorbance will also be explored.

PROCEDURE

Sample Preparation

1. Place about 5 mL of copper (II) sulfate (0.015M) into a clean labeled spectrophotometer tube. Add 5 drops of concentrated ammonia (NH_3) to the tube and mix carefully.
   The following reaction occurs on mixing:

   $$Cu^{++}(aq) \quad + \quad 4\ NH_3(aq) \rightarrow Cu(NH_3)_4^{++}(aq)$$

   If any white precipitate, $Cu(OH)_2$, remains add 1 or 2 drops more of ammonia to dissolve it.

2. Prepare a dilution of the 0.015M $CuSO_4$ by placing 5.0 mL of 0.015M $CuSO_4$ into a 25 mL graduated cylinder and adding water to bring the total volume to 15.0 mL. Mix the solution. Calculate the concentration of $CuSO_4$. Place 5 mL of this dilution into a clean labeled spectrophotometer tube and add 5 drops of concentrated ammonia. Mix well.

   Repeat using 10.0 mL of $CuSO_4$ and diluting to a final volume of 15.0 mL. Calculate the concentration of $CuSO_4$. Place 5 mL of this dilution into clean labeled spectrophotometer tubes and add 5 drops of concentrated ammonia. Mix well. Save the unused 10 mL of this solution for Part II and add 10 drops of concentrated ammonia to it.

3. Prepare a blank for calibration by placing 5 mL of distilled water into a clean labeled spectrophotometer tube and adding 5 drops of concentrated ammonia. The blank contains all the same reagents except the analyte. Any absorbance due to water (solvent), reagents, and/or the glass spectrophotometer tubes will be removed when the blank is set to zero absorbance (or 100%T) during calibration.

Part I- Effect of Wavelength

4. Using the Spec-20D and the instructions given by the computer for the "A vs λ" function, scan the 0.015M Cu-ammonia complex solution from 400 to 650 nm at 20 nm intervals. Locate the wavelength of maximum absorbance, λ_{max}, from the plot on the computer screen. Generate/print a graph of absorbance vs. wavelength from the computer.

Part II- Effect of Pathlength

5. One of the Spec-20D instruments will be set up to change the size of the sample tubes used. Set the wavelength control to λ_{max} as determined in Part I. Measure the absorbance for the 0.010M Cu-ammonia complex solution in the three different sized tubes provided. **Your instructor will show you how to set up the instrument and how to change the sample tube holders.** Record the data in the section provided. Generate/print an absorbance versus pathlength graph. Determine the regression equation (trendline) and r^2 value and be sure these are printed on the graph.

Part III- Effect of Concentration

6. Measure the absorbance of 0.005M, 0.010M, and 0.015M Cu-ammonia complex solutions at λ_{max} using the "A vs. C" function. Record the data and generate/print an absorbance versus concentration graph (calibration curve). Determine the regression equation (trendline) and r value and be sure these are printed on the graph. Change the wavelength from λ_{max} to 550 nm and repeat the measurements for the three Cu solutions. Record the wavelength and absorbances. Generate/print an absorbance versus concentration graph. Determine the regression equation (trendline) and r^2 value and be sure these are printed on the graph.

Part IV- Potential Errors

7. Place the 0.015M Cu solution in the sample holder with the mark on the spectrophotometer tube aligned with the mark on the sample holder. Close the lid and read the absorbance.

   Consider each of the actions below. In the Data section, predict and record what might happen to the absorbance if this action was taken before making an actual measurement. Once you have recorded the prediction, read and record the absorbances at λ_{max} for each situation:

   - Leave the lid open;
   - Rotate the tube 45^o (quarter turn);
   - Remove the tube and place 20-30 fingerprints on it;
   - Clean the tube of fingerprints and add a small amount of Fuller's Earth and mix.

DATA

Part I- Effect of Wavelength (using 0.015M Cu solution)

Wavelength	Absorbance	Wavelength	Absorbance
400 nm		540	
420		560	
440		580	
460		600	
480		620	
500		640	
520		650	

From computer plot λ_{max} = _________.

Part II- Effect of Pathlength (using 0.010M Cu solution)

Pathlength	Absorbance
0 cm	0
1.2 cm	
1.8 cm	
2.4 cm	

Part III- Effect of Concentration

Concentration	Absorbance at λ_{max}	Absorbance at 550 nm
0 (blank)		
0.005M		
0.010M		
0.015M		

Part IV- Potential Errors

Potential Error	Prediction	Absorbance
None (control)	----------------------	
open lid		
rotated tube		
fingerprints		
Fuller's Earth		

RESULTS

Generate the following graphs and attach them along with any calculations. For each graph generate the best fit curve or plotted regression equation (a smooth curve or straight line) through the data using the appropriate function in SpectroPro or Excel. Be sure that the regression equation and r^2 value are displayed on the graph in the form of the variables.

- absorption spectrum (absorbance vs. wavelength)
  locate λ_{max} on the graph
- absorbance vs. pathlength
  determine the slope
- calibration curve (absorbance vs. concentration)
  (plot both wavelengths using different symbols)
  determine the slope for each wavelength

CONCLUSIONS

1. Describe the type of relationship found for each of the three variables explored and absorbance. If the wavelength is held constant, write a simple mathematical statement of the incorporating absorbance, pathlength, and concentration.

2. Explain the cause of each of the errors examined in part IV.

Error	Possible Cause
open lid	
rotated tube	
fingerprints	
Fuller's Earth	

POST-LAB QUESTIONS

1. On the absorption spectrum of the 0.015M Cu-ammonia complex solution, sketch as a dashed line the curve for a 0.005M Cu solution.

2. If the ammonia was **not** added to the copper (II) sulfate solutions, how would the results have been influenced? Explain.

3. What happens to the concentration when the cell size (pathlength) is doubled? Explain.

4. Beer's law, stated mathematically, is given below:

$$A = abc$$

where the terms are defined as A = absorbance; a = absorptivity; b = pathlength; and c = concentration (if c = molar concentration then a = molar absorptivity which is sometimes abbreviated with ε).

The equation for a straight line is y = mx + b, where m is the slope and b the y-intercept. If the line passes through the origin, then b is zero and the equation simplifies.

What type of plot did you get for A vs. b? What is the slope equal to?

What type of plot did you get for A vs. c at λ_{max}? What was the slope equal to?

How did the slope for A vs. c at λ_{max} compare to the slope for the same graph at 550 nm? Explain why.

5. Calculate the molar absorptivity at λ_{max} for $Cu(NH_3)_4^{++}$ using the slope of the calibration curve and Beer's Law.

6. What variable in Beer's Law is changing to cause the difference in slope for the two calibration curves determined in part III?