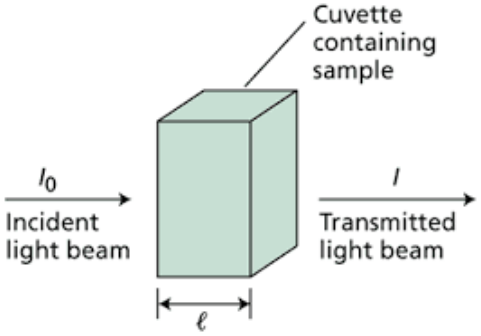
Name: _____ Partner: _____

Spectrophotometric Analysis: Concentration of a Copper Sulfate Solution Using Beer's Law

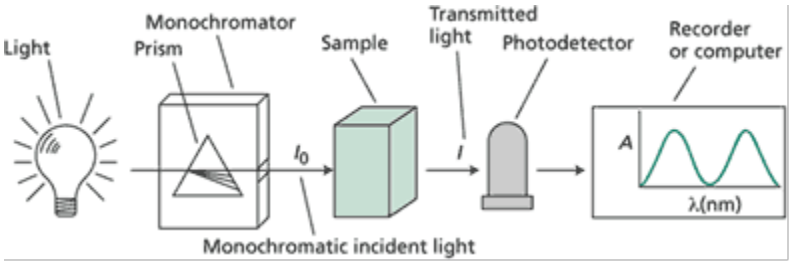
Purpose: The purpose of this lab is to determine the concentration of an unknown Copper Sulfate solution.

Introduction:
When white light is allowed to pass through a prism, the light is refracted into a spectrum of colors. This spectrum of colors has a range of wavelengths varying from about 400 nm (violet) to about 800 nm (red) and is referred to as the visible spectrum. Light of a single wavelength is called monochromatic light. When monochromatic light passes through a solution sample of uniform thickness, part of the light is absorbed and part of the light is transmitted (see figures below). Beer's Law states that the absorbance of light by a solution is directly proportional to the concentration.

A single wavelength of light is shone on the sample. The wavelength chosen is a color that is complementary to the color of the solution.



The solution absorbs some of this light. The amount of light that is transmitted can be used to calculated the % **transmittance** (T) by I/I_o. Or the **absorbance** (A) can be calculated by
2 – log % T



Components of a Spectrophotometer
1. Stable source of radiant energy. A tungsten filament source is useful in the visible region.
2. Monochromator – Limits radiation to a particular narrow band of wavelengths.
3. Nonabsorbent containers of constant thickness for the sample.
4. Photodetector to convert radiant energy to an electrical signal.
5. Meter or other signal detector.

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Beer's Law can be applied to the solution provided no change, other than a change in concentration, occurs in the species responsible for the light absorption in the solution. The solution follows Beer's Law according to the following equation:

$$A = \varepsilon b c$$

where

ε = molar absorptivity, which depends on the substance, the solvent and the wavelength, λ.
b = path length of the light through the solution (also called the cell length), usually 1.00 cm.
c = molar concentration

The molar absorptivity, ε, depends on the substance, the solvent, and wavelength, λ.
The units for molar absorptivity are $1/M \cdot cm^{-1}$ or $L/mole \cdot cm^{-1}$.

PLAIN ENGLISH: The amount of light at a given wavelength that is absorbed by a solution depends on the concentration of the solution, the thickness (diameter) of the solution, and a constant. OR the concentration of the solution is directly proportional to the absorbance.

Materials: spectrophotometer, test tube rack, 8 disposable cuvettes, 2 handheld pipettes, 2 beakers, 5 volumetric flasks, distilled water,.4 molar Copper Sulfate stock solution.

Part 1: Determining the Wavelength of Maximum Absorbance

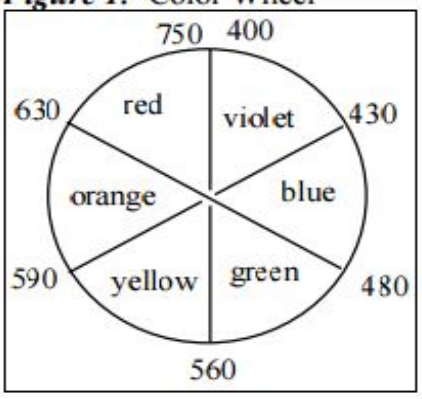
A color wheel illustrates the approximate complementary relationship between the wavelengths of light absorbed and the wavelengths transmitted. For example, a red substance would absorb the complementary (opposite it in the color wheel) color of light, green. When light is not absorbed, it is said to be transmitted through the solution. The wavelengths that a substance absorbs can be determined by exposing the solution to monochromatic light of different wavelengths and recording the light transmitted.

Determine what wavelength of light this compound absorbs most. Based on the color of the solution what do you predict it will be?

Table 1: Absorbed & Perceived Colors

Absorbed Wavelength (nm)	Absorbed Color	Perceived (Transmitted) Color
400	violet	green - yellow
450	indigo	yellow
480	blue	orange
490	blue-green	red
530	green	purple
570	yellow-green	dark blue
600	orange	blue
650	red	green

Figure 1: Color Wheel



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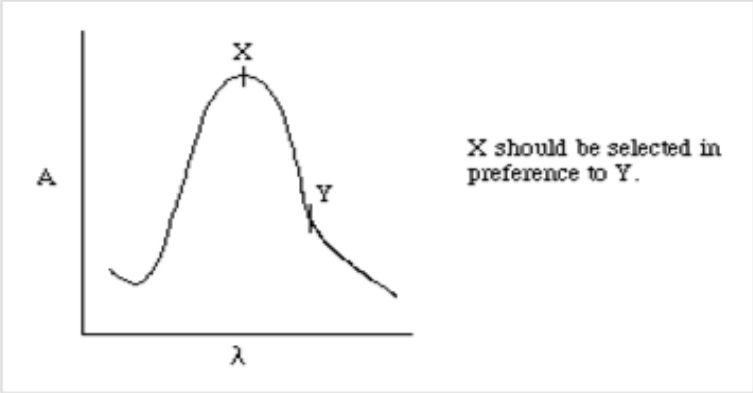
Spectral Curve

1. Your instructor will show you the correct use of a spectrophotometer. Each model is slightly different in the way solutions are measured.
2. Pipette some of the solution you have made into a cuvette until the cuvette is about 80% full of the solution. Wipe the outside (with Kim wipes) so that no fingerprints are on the cuvette. Try to only touch the top of the cuvette.
3. Carefully place the cuvette into the spectrophotometer
4. Measure the **ABSORBANCE** (make sure it is set to read absorbance, not transmittance) every 20 nanometers from 440–660 nm. Place this information on a data table. If the absorbance starts flashing, then don't worry about that data point. You are now at a wavelength at which absorbance cannot be measured.

DATA TABLE 1

Wavelength	Absorbance	Wavelength	Absorbance

5. Construct a plot of absorbance vs. wavelength, and determine the maximum wavelength This max wavelength will be used for the rest of the lab



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Part 2: Determining the Relationship between the Concentration and Absorbance

1. Use a 10 mL graduated pipet and/or graduated cylinder to measure the required volumes of 0.400M solution (from the blue Copper Sulfate stock solution) and H_2O into a volumetric flask as indicated below. Do NOT attempt to mix each solution in the cuvette as it will not hold the entire solution. Swirl each solution in the volumetric flask. Place each solution into a cuvette – but only enough to fill it to about 80% of the way. **Remember to wipe the outside (with Kim wipes) so that no fingerprints are on the cuvette. Try to only touch the top of the cuvette.**

DATA TABLE 2

Vol. 0.400 M solution (mL)	Vol H_2O (mL)	Concentration (molarity)	Absorbance
2.00	8.00		
4.00	6.00		
6.00	4.00		
8.00	2.00		
10.00	0		
Unknown	X		

2. BE VERY CAREFUL CARRYING THE CUVETTES TO THE SPECTROPHOTOMETER. This is when we break cuvettes every year.
3. Measure the **absorbance** of each of these solutions at the maximum wavelength you chose in part 1 (you will not be changing the wavelength any more). Yes, just stick them in and read the **absorbance**!
4. **Take a picture of the 5 solutions you made**. Make sure the color of your test tubes/ cuvettes are easily seen in the picture.

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Part 3: Determining the concentration of an unknown sample.

1. Obtain a solution of unknown concentration from your instructor.
2. Look at your 5 known solutions from the last part and compare to the unknown using your eyes, GUESS which 2 solutions it is in between according to concentration. What do you expect its absorbance to be?
3. Measure the absorbance of this diluted solution at the wavelength of maximum absorbance.

Calculations:

1. Show a sample calculation for finding the molarity.

2. Construct a Calibration Curve by plotting absorbance vs concentration from data Table 2 (use sparkvue, excel or google sheets)

3. From the graph determine the equation for the line of best fit and determine the meaning of the slope (hint look at the Beer's Law Equation). Why should the y-intercept be zero?

4. Determine the concentration of the unknown solution by using the equation, using the absorbance measured.

5. Using the picture you took earlier, how close were you with your unknown prediction?

6. Calculate your percent error for the concentration of the unknown assuming the unknown was _____ (ask your teacher for this)

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Questions: Use complete sentences and explain fully.

1. What is Beer's Law? Describe it completely in words and in an equation! State what each variable is. (Use the version of Beer's Law explained in THIS lab handout)

2. In the version of Beer's Law, $A = \varepsilon bc$, what variables were held constant in this lab? Which one was not?

3. Spectroscopy is the use of energy to determine properties of a substance. In this case, we used visible light to discover what property about our substance?

4. What wavelength of light did you pick to use in this lab? Why?