

Using the Visible Light Spectrophotometer to Determine the Concentration of an Unknown Solution

Objective:

- The absorbance or transmittance of light at a given wavelength is an indication of a molecule's presence in solution
- The concentration of molecules in a solution affects the solution's absorbance

Background: In the electromagnetic spectrum, visible light ranges from 400 nm to 700 nm. Radiation is the result of electrons moving from one energy level to another. In order to move to a higher energy level, they have to absorb energy (light radiation). The absorption of light results in color in solutions of transition metal complexes.

The spectrophotometer will be used to measure the intensity of light entering a sample, the amount of light exiting a sample, and compares the two intensities. The two intensities can be expressed as transmittance (the ratio of the intensity of existing light to entering light), or percent transmittance (%T). Since different materials absorb different wavelengths of light, a material can be identified by the wavelength of maximum absorption. Once the wavelength has been identified for a material, concentration (Molarity), of the solution can be determined using a standard curve of known concentrations.

Beer's law states the absorbance is directly proportional to the concentration of a solution. If you plot the absorbance (y-axis) versus the concentration (x-axis) you can determine the concentration of an unknown solution using the graph or by using the equation for a line.

Materials:

- VIS spectrophotometer
- 0.2 M CuSO₄
- 0.4 M CuSO₄
- 0.6 M CuSO₄
- 0.8 M CuSO₄
- 1.0 M CuSO₄
- One unknown concentration of CuSO₄
- Spectrophotometer test tubes (13x100 glass)
- Pipettes for Measuring Solutions

Procedure:

1. Turn on the spectrophotometer and allow it to warm up for 20 minutes
2. Blank the spec according to manufacturer's instructions using a wavelength of 520 nm
3. Set the mode to absorbance for data collection
4. Insert one known sample into the chamber
5. Record the absorbance value in the data table
6. Repeat steps 4 and 5 using the other known samples
7. Graph the data below. Put the molarity on the x-axis and the absorbance on the y-axis
Biotech Data Hint: (Always include a graph title that includes both the IV and DV, as well as axes titles with the units used. YES, the data table and graph may have the same title!)
8. Create a standard curve with the data points
9. Insert the unknown solution into the chamber
10. Using your graph and the standard curve, determine the molarity of the unknown solution

Before placing a tube into the spec, always wipe the tube with a KimWipe® to remove fingerprints or dust.

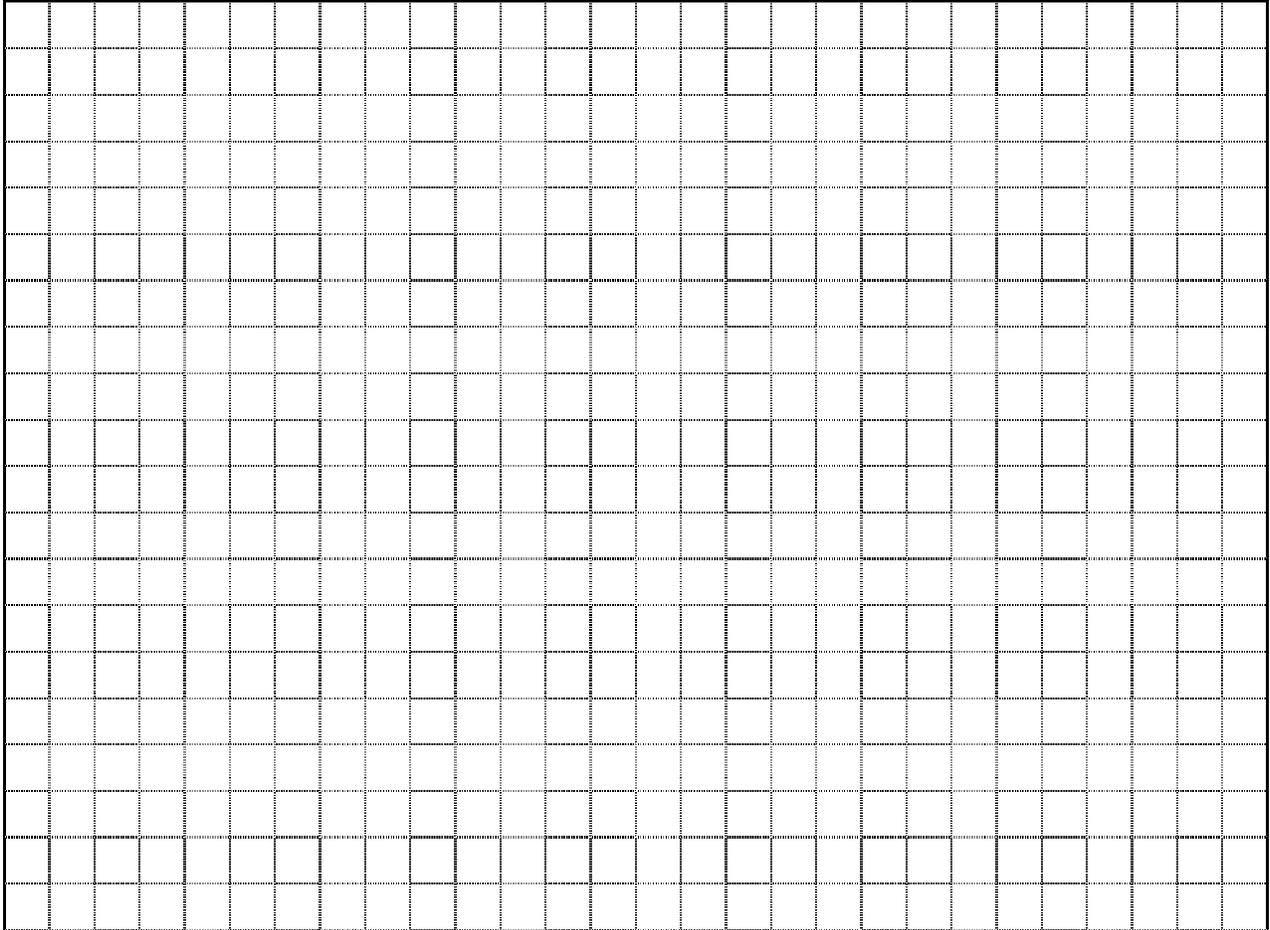
Data table:

Affect of Copper Sulfate Molarity on the Absorbance of Light

<i>Molarity (mol/L)</i>	<i>Absorbance (au)</i>
0.2	
0.4	
0.6	
0.8	
1.0	
Unknown	

Biotech Data Hint: (Always add data from smallest to largest or alphabetically)

Graph:



Unknown Molarity _____

Questions:

1. What is the independent variable and dependant variable for this lab?
2. Why is the absorbance value increasing with an increase of concentration?
3. If the wavelength is changed, what will happen to the absorbance value?
4. What is a real world application for this procedure?
5. Please obtain your actual molarity of your unknown solution from the instructor and determine the percent error for the lab.