SPECTROSCOPY: BEER'S LAW

INTRODUCTION

A useful analytical tool for determining the concentration of colored material in solution is absorption spectrophotometry. Colored substances absorb light in the visible spectrum and the amount of light absorbed is proportional to the concentration of the substance in solution.

If I_o is the intensity of light entering a solution and I_t is the intensity of light exiting the solution, then the transmittance, T, of the solution is given as I_t/I_o. Transmittance is also expressed as a percentage, $(I_t/I_o)(100\%)$. Frequently, the absorbance, A, rather than transmittance is used for the amount of light a solution absorbs. Absorbance is defined by the equation $A = -\log(T)$ or $A = \log(I_o/I_t)$. The absorbance of a solution depends on the quantity of light absorbed by the species in the solution, the wavelength of the light entering the solution, the length of the solution the light has to pass through, and the concentration of the solution. This relationship is known as Beer's law and is expressed mathematically as $A = abc$. Here "a" is the proportionality constant (molar absorptivity if concentration units are molarity), "b" is the path length of radiation going through the solution, and "c" is the concentration of the solution.

In this experiment an absorption spectra curve of absorption versus wavelength will be obtained for a cobaltous nitrate hexahydrate $Co(NO_3)_2 \cdot 6H_2O$ solution. From the absorption spectra, the wavelength of maximum absorption is determined and a calibration curve relating absorption to concentration for cobaltous nitrate is prepared. Using the calibration curve, the concentration of an unknown cobaltous nitrate solution is determined by measuring its absorption at the selected wavelength.

PROCEDURE

Solution Preparations

Prepare 10 mL of 0.100 M $Co(NO_3)_2 \cdot 6H_2O$ by weighing 0.291 g of the salt into a 10.00 mL volumetric flask. Add some water and dissolve the salt. Dilute the solution to the mark with water.

Prepare a 0.0500 M solution of the salt by pipetting 5.00 mL of the 0.100M solution into a clean 10 mL volumetric flask and dilute to the mark with water. Pipette 5.00 mL of the 0.0500M solution into a clean 10 mL volumetric flask and dilute with water to the mark to give 0.0250M salt. Prepare 10.00 mL of 0.0125 M solution by pipetting 5 mL of the 0.0250 M solution into a clean 10 mL volumetric flask and dilute to the mark with water.

Absorption Spectra Procedure

Record your partners' names. You must each record the data. Following your instructor's directions, calibrate your spectrophotometer so it reads 0% T with no cuvette in the cell compartment. Set the wavelength to 400 nm. Put some distilled water into a clean dry cuvette and place it into the cell compartment. Using the light current knob adjust the % T to read 100 %. Put some of the 0.100 M cobalt solution into a clean dry cuvette. Insert the cuvette containing the 0.100 M cobalt solution into the cell compartment and measure its absorbance. Change the wavelength to 410 nm. Calibrate the 100% T with distilled water and measure the absorbance of the 0.100 M solution. Repeat this process at 10 nm intervals up to 600 nm. From the above data determine the wavelength of maximum light absorption.

Calibration Curve Procedure

Set the wavelength of your spectrophotometer to the wavelength of maximum absorption as determined in the preceding procedure. Calibrate the 100 % T at this wavelength using distilled water. Now recheck the absorbance of the 0.100 M solution. Measure the absorbance of the 0.0500M, 0.0250 M, and 0.0125 M standard cobalt solutions at the selected wavelength.

Unknown Solution

Obtain a vial of cobalt solution of unknown molarity and measure its absorbance at the selected wavelength used above.

DISPOSE ALL COBALT SOLUTIONS IN THE HEAVY METAL CONTAINER.

Students may work in pairs on the absorption spectra and the calibration curve but **EACH STUDENT MUST DO THEIR <u>OWN</u> UNKNOWN.**

Name:_____ **Partner:**_____

Absorbance Data

Wavelength (nm)	Absorbance
400	
410	
420	
430	
440	
450	
460	
470	
480	
490	
500	
510	
520	
530	
540	
550	
560	
570	
580	
590	
600	

Wavelength of maximum absorption: _____nm

Name:_____ **Partner:**_____

Calibration Curve Data

$[Co(NO_3)_2 \cdot 6H_2O]$ (M)	Absorbance
0.000	0.00
0.0125	
0.0250	
0.0500	
0.100	
Unknown # _____	

RESULTS

Absorption Spectra

By hand or using Excel or Graphical Analysis, plot a graph of absorbance vs. wavelength for the 0.100 M cobalt solution. For this graph, it is okay to leave the connecting line in ("connect-the-dots"). Include the graph with the report.

Calibration Curve

Using Excel or Graphical Analysis, plot a graph of absorbance vs. concentration for the standard cobalt solutions. Fit the best straight line to the data and obtain the slope of the straight line. The y-intercept, b, should be approximately zero. Include the graph with the report.

From the calibration curve and the absorbance of your unknown, determine the molarity of your unknown solution. Remember the following.

A = abc or A = mc (where m is slope of the calibration curve)

Slope of Calibration Curve _____

Unknown Solution Molarity _____

Name:_____

Spectroscopy: Beer's Law

PRESTUDY

1. How many grams of cupric sulfate pentahydrate are needed to prepare 50.00 mL of 0.0800M $CuSO_4 \cdot 5H_2O$?

2. Indicate how you would prepare 50.00 mL of 0.0400M $CuSO_4 \cdot 5H_2O$ from the above cupric sulfate solution.

3. The following data were obtained for $CuSO_4 \cdot 5H_2O$ solutions at a wavelength of 650 nm.

Concentration (M)	Absorbance
0.000	0.000
0.0100	0.110
0.0200	0.200
0.0400	0.450
0.0800	0.920

a. Using Excel or Graphical Analysis, plot a graph of absorbance (y-axis) vs. concentration (x-axis) using the above data. Get the best straight line and the slope of this line. Include the graph with the prestudy.

b. From the graph determine the molarity of a copper sulfate pentahydrate solution whose absorbance is 0.170 at 650 nm.