Spec-20 & Beer's Law Lab

Theory & Background

Many compounds absorb light from region of the electromagnetic spectrum. A *spectrophotometer* is a device designed to determine the wavelengths if light that a compound absorbs. When an aqueous sample of a compound is placed in the light path of a spectrophotometer, the sample may absorb all the light, some of the light, or no light at all. The absorption of light depends upon the materials in the sample and the wavelength of the light. Light absorption occurs at wavelengths whose energy corresponds to the energy necessary to cause electronic excitation ns of atoms, ions, or molecules in the sample. From the spectrophotometer data, a graph can be made that plots the light intensity transmitted through the sample versus the wavelength of the light; such a graph is called an *absorption spectrum*. The range of wavelengths absorbed by the sample appears as bands of minimum intensity.

Absorption spectra are useful for two reasons. First, the absorption spectrum of a substance is a unique characteristic of that substance. This makes the spectrum useful for the identification of unknown substances. Second, the intensity of the absorption bands can be related to the concentration of the substance in the sample. Thus, the intensity of the absorption band can be used to determine the amount of a particular substance in a mixture.

Beer's Law Information: http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/beers1.html

Purpose:

To familiarize you with the Spectronic 20, a commonly used machine for Chemistry and Biology, and how to calibrate and operate it. In Part I, To learn to make dilutions and to calculate their molarity. To determine unknown concentrations by results from the Spec 20 and from a graph of percent transmittance and molarity. In Part II, you will determine the absorption spectrum of an aqueous solution of chromium (III) ions.

Materials:

0.400 M $CuSO_4$ solution, 2 - 10 mL volumetric pipettes, pipette bulb, Spectronic 20 tubes or cuvettes, , 2 - test tube racks, Kimwipes/tissues, a 50 mL beaker, 100 mL beaker, 1 -unknown concentration tube, water blank, distilled wash bottle, and a Spectronic 20. For the second part, you'll need 0.02 M **$Cr(NO_3)_{3(aq)}$**

Safety: Chemicals ($CuSO_4$ & $Cr(NO_3)_3$) can be an irritant to skin, avoid contact, wash immediately and then contact instructor for further first aid.

• Always wash your hands with soap and water before leaving lab.

PRELAB: All your prelab questions can be answered from this website (What's in the Mix? Lab):
http://kicp-yerkes.uchicago.edu/2004-summer/pdf/ysi2004-liquid-colors.pdf
Please write these questions and your answers in your lab notebook.

1. What are three things that spectroscopy can tell us?
2. How is spectroscopy used to identify a substance?
3. How does a spectrophotometer work? Follow white light through the device, describe the parts that it passes and what they do.
4. What is a blank? How much transmittance of light is present?
5. What is Beer's Law and what does it tell you?

(http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/beers1.htm)

Procedure:
Part I --- $CuSO_4$

1. You are now ready to calibrate the Spec 20. First prepare a *blank* by filling a cuvette 3/4 full with distilled water. To correctly use a colorimeter cuvette, remember:

- All cuvettes should be wiped clean and dry on the outside with a tissue.
- Handle cuvettes only by the top edge of the ribbed sides.
- All solutions should be free of bubbles.
- Always position the cuvette with its reference mark facing toward the white reference mark at the right of the cuvette slot on the colorimeter.

To calibrate the cuvette at 0% and 100% transmittance: Place the blank cuvette in the cuvette slot of the Spec 20 and close the lid. Push the green button (100%T or 0%A). Set the wavelength knob to 635 nm. n this position, the colorimeter is calibrated to show 100% of the RED light being transmitted through the blank cuvette.

2. Next you will find the %T for your tubes from the Spec 20. You will also find the %T for the unknown at this time.

3. Pipet 2, 4, 6, and 8 mL of 0.40 M $CuSO_4$ solution into Test Tubes 1-4, respectively. With a second pipet, deliver 8, 6, 4, and 2 mL of distilled water into Test Tubes 1-4, respectively. *Thoroughly* mix each solution with a stirring rod. Clean and dry the stirring rod between stirrings. Keep the remaining 0.40 M $CuSO_4$ in the 100-mL beaker to use in the fifth trial. Volumes and concentrations for the trials are summarized below:

Trial Number	0.40 M $CuSO_4$ (mL)	H_2O (mL)	Concentration (M)
1	2	8	0.08
2	4	6	0.16
3	6	4	0.24
4	8	2	0.32
5	~10	0	0.4

DATA AND CALCULATIONS

Trial	Concentration (mol / L)		Absorbance
1	0.080		_____=
2	0.16		_____=
3	0.24		_____=
4	0.32		_____=
5	0.40		_____=
6	Unknown number	_____ =	_____=
Slope of regression line (m)			_____ L/mol
Concentration of the unknown			_____ mol/L

GRAPH 1: BEER'S LAW

4. With a different pen/pencil color, circle on your graph where your unknown absorbance crosses your line. Also write in what the concentration is at those point from the graph, using the axis **(NOT YOUR CALCULATED VALUES)**. *(Make a key for the colors)*

Procedure:

Part II --- $Cr(NO_3)_3$

1. Turn on the spectrophotometer and allow it to warm up for about 20 minutes.

2. Set the wavelength control knob to 375 nanometers (375 nm). Adjust the amplifier control knob to produce 0 percent transmittance (0%T) at this wavelength.

3. Add 3mL of distilled water to a cuvette.. Wipe the outside of the tube with a tissue to make certain that it is clean and dry. Avoid getting fingerprints on the tube. Dislodge any air bubbles present in the water by gently tapping the tube with a finger.

4. Place the tube in the sample holder and close the cover. Adjust the light control knob until the spectrophotometer reads 100%T

5. Remove the first sample from the spectrophotometer. Add 3mL of 0.02*M* chromium (III) nitrate, $Cr(NO_3)_3$, to another clean test tube. Use a tissue to clean and dry the tube. Insert the cuvette of chromium (III) nitrate into the sample holder. Read the percent transmittance and record the reading in Data Table 2. Remove the sample from the holder.

6. Turn the wavelength dial to 400 nm. Use the amplifier control knob to adjust the percent transmittance to 0%T. Place the water sample in the holder. With the light control knob, adjust the meter to 100%T. Replace the water sample with the chromium (III) nitrate sample. Measure and record the percent transmittance at 400 nm.

7. For the remainder of the wavelength listed in Data Table 2, continue the procedure of setting 0%T, setting 100%T, and measuring the percent transmittance of the chromium (III) nitrate solution.

8. Unless directed otherwise by you teacher, return the aqueous chromium (III) nitrate to the dropper bottle.

Observations-DATA TABLE2: PERCENT TRANSMITTANCE AND ABSORBANCE OF 0.02*M* $Cr(NO_3)_3$ SOLUTIONS AT VARIOUS WAVELEGTHS

Wavelength (nm)	% Transmittance (%T)	Absorbance
375		
400		
405		
415		
425		
440		
455		

470		
490		
500		
520		
530		
540		
550		
570		
575		
580		
600		
625		

DISCUSSION/CALCULATIONS

1. **Graph 2: percent transmittance versus wavelength.** The curve you plot is the absorption spectrum of chromium (III) ions in the visible region of the electromagnetic spectrum.

2. At what wavelengths do chromium (III) ions absorb the maximum amounts of light? What colors of light correspond to these wavelengths?

3. Based on the answer to problem 2, would you expect a red solution to absorb or transmit red light? Explain.

4. The amount of light that is absorbed by a solution is commonly expressed either in terms of percent transmittance, as in this experiment, or in terms of absorbance (A).

Absorbance is defined as: 2 – log of percent transmittance OR $A = 2 - \log \%T$

Given the relationship shown in the preceding formula, convert the percent transmittance values in Data Table 1 to absorbance values. **Graph 3**: **Plot a graph of absorbance versus wavelength.**