Experiment 3: Introduction to Spectroscopy

Chemistry 113 Lab Preparation Form

Name_____ Lab Section_____

Purpose of Experiment:

Objectives: To determine the concentration of dye in an unknown solution using spectroscopic technique.

Key Terms:

Spectroscopy

Absorbance

Transmittance

Beer's Law

Calculations:

Safety Warnings:

Procedure Notes:

Questions before starting experiment?

Comments from Briefing:

Experiment 3: Introduction to Spectroscopy

Prelab Exercise

Name_____ Lab Section_____

1. Identify the terms in the Beer's law equation.

2. A sample gives a %transmittance of 26. What is its absorbance?

3. The sample in question 2 has a concentration of 0.121M. The sample has a pathlength of 1.00cm. What is the absorptivity of the sample? (remember to include units!!)

4. If the sample in question 3 is diluted to a concentration of 0.0121M, what will be the new absorbance? Note: pathlength and absorptivity are constant.

Experiment 3: Introduction to Spectroscopy

Introduction:

Spectroscopy studies the interaction of light with matter. Colored analytes (species being studied) absorb light at specific wavelengths. When light of an appropriate wavelength passes through a sample, the emerging light is of lower intensity that that entering the sample (see Figure 1).

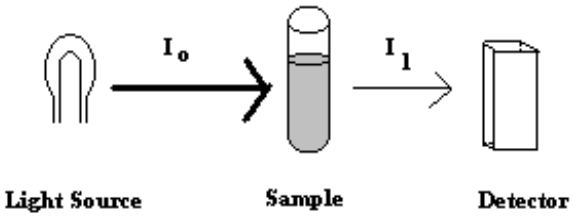


Figure 1

Where I_o is the intensity of the incident light and I_1 is the intensity of the emerging light.

The ratio of I_1/I_o is called the Transmittance. The Absorbance (A)is equal to the negative log of the ratio (I_1/I_o) or {$A = -\log(I_1/I_o)$}. The absorbance is proportional to three factors:

a : Absorptivity of the sample
b : Distance the light travels through the sample
c : Concentration of the absorbing species

The relationship is expressed as **A = abc** and is known as Beer's Law.

The Spectronic™ 20D is an instrument that measures the % transmittance of light at various wavelengths. The absorbance is found using A=-log(%T/100). As can be seen from Beer's Law there is a direct relationship between the Absorbance, A, and the Concentration, c, of the sample. If the values of the constants a and b are known, the concentration of a solution may be determined from its absorbance of light. Typically, a series of solutions of known concentrations are prepared and the absorbance of each of these solutions is measured. A graph of absorbance versus concentration should give a straight line with a slope of ab. Once the data are plotted, the graph can be used to determine the concentration of a solution for which the absorbance has been measured. This is done using a best-fit line.

GRAPHING:

Construct a graph of Absorbance versus the concentration of dye in the liquid. You are required to use the computer to generate this graph. Your lab instructor will help you during lab to create the graph. You can also get their help during their office hours. ***Important!*** Proper graphing technique includes the following:

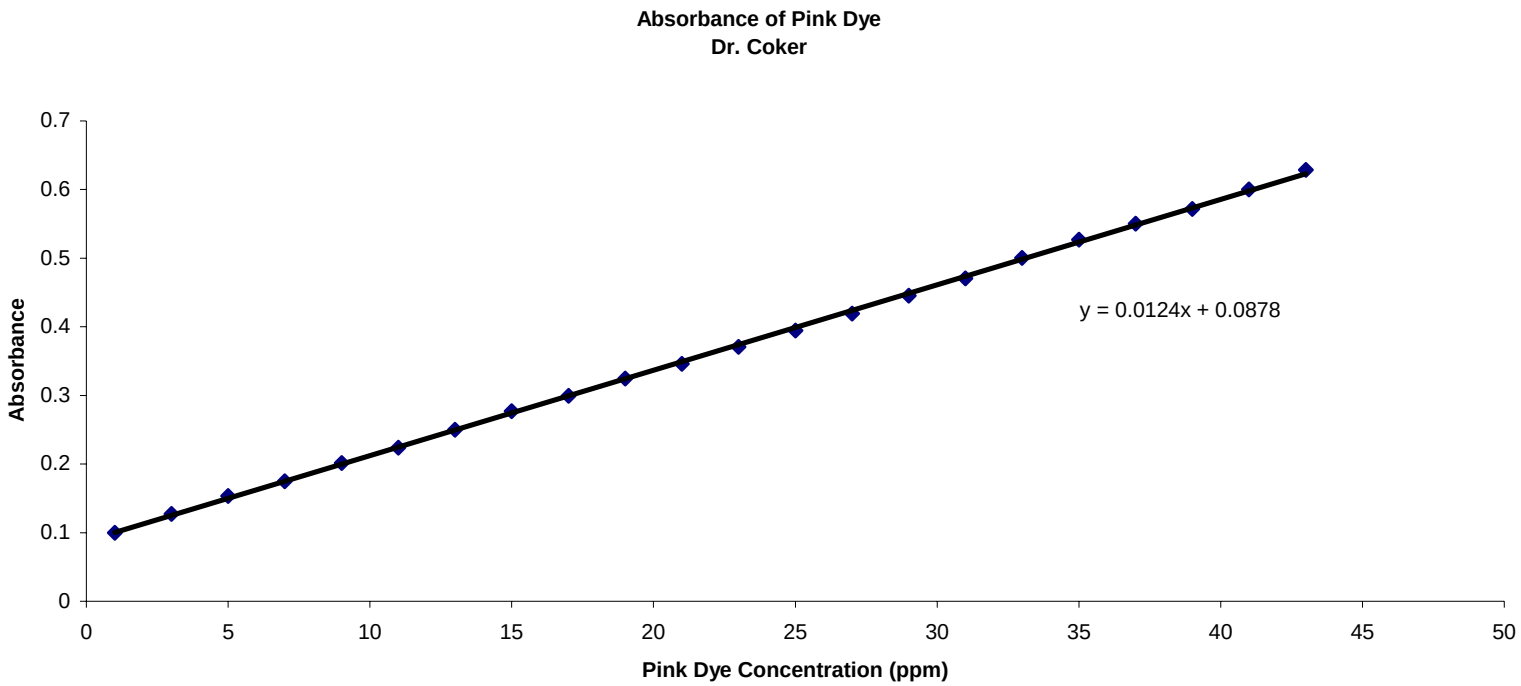
1. A specific title with your name as a subtitle.
2. Each axis is labeled.
3. Units are identified on each axis.
4. A best line is drawn; i.e., do not just connect data points.
5. Spread the graph out to fit as much of the page as possible.

Instructions for graphing using Microsoft® Excel.

1. Open the Microsoft® Excel Program.
2. The program should default you to cell A1. This is column A and Row 1. Starting with this cell and going down the A column, enter your X values (Concentration in this case). Do not include Units. Now go to cell B1 and going down that column enter the corresponding Y values (Absorbance in this case). Do not include Units.
3. Highlight your data set and select the red, yellow and blue "Chart Wizzard".
4. Select XY scatter and then select next. Select next again.
5. Add a descriptive Title. Remember to Capitalize first letters of main words. Add a few spaces after the title and type your name.
6. Add a title for the X and Y-axes. Include an abbreviation for the units inside parenthesis.
7. Select the Gridlines tab. Remove the gridlines.
8. Select the Legend tab. Remove the legend.
9. Select next.
10. Place the chart as a new sheet and select finished.
11. Click on the title just before your name. A cursor will appear. Press enter. This will place your name below the title. Highlight the title and make it a larger fontsize.
12. Right Click on the gray background of the graph and select clear. This will remove the gray background.
13. Move the cursor to one of your data points. Right click. Select add trendline. Under options select display equation on chart. Click ok.
14. If your graph looks ok, print it.

An example graph is shown in Figure 1. Remember that your graph should fill the page.

Figure 1: A Sample Graph



Your graph should fill the entire page. You are required to have a graph done using a spreadsheet program. The absorbance is for the solution that is placed into the Spectronic™ 20D. You will be asked to give the concentration of an unknown solution. You might have had to dilute the unknown solution so remember to take this into account. You are ultimately asked the concentration of dye in the unknown, not the concentration of dye that you placed into the machine.

EXPERIMENTAL: (This procedure will be repeated for two dyes.)

Work in groups: Using a clean dry beaker, obtain about 75 mL of known dye solution. Write down the name of the dye and its concentration. Rinse a clean 50 mL burette with a small amount of the dye solution. Fill the 50 mL burette with the dye solution. Remember to check for air bubbles.

Using a clean dry beaker, obtain about 75 mL of distilled water. Rinse a clean 50 mL burette with a small amount of the distilled water. Fill the 50 mL burette with distilled water. Remember to check for air bubbles.

Obtain 10 beakers and label them 1 to 10. Approximately fill each beaker according to the table below. Record the exact volumes used.

Beaker #	mL of known dye solution	mL distilled water
1	25.00	5.00
2	25.00	5.00
3	25.00	5.00
4	15.00	10.00
5	15.00	10.00
6	15.00	10.00
7	5.00	15.00
8	5.00	15.00
9	5.00	1500
10	0.00	0.00

Obtain 12 cuvettes. Label them 1 to 9. Fill cuvettes 1 through 9 with the solutions from their respective beakers. Fill cuvette 10 with distilled water. Fill cuvette 11 with unknown solution. Write down the unknown number.

Calibrating the Spectronic™ 20D:

Make sure the Spectronic™ 20D is set for the appropriate wavelength. The appropriate wavelength is written on the standard bottles.

Set the 0 % transmittance with nothing in the Spectronic™ 20D. Do this using the 0% Transmittance knob and adjusting until the reading is 0. Then place a wiped cuvette full of distilled water into the sample chamber (cuvette 11). Make sure the mark on the cuvette is aligned with the mark on the Spectronic™ 20D.

Set the Spectronic™ 20D to read 100 using the 100 % transmittance knob.. Remove the cuvette and make sure that the 0 transmittance has not changed. This is

calibrating the instrument and will be done between each reading. Now the Spectronic™ 20D is ready to take 1 reading.

Record the % transmittance of each cuvette and that of the unknown. Remember to recalibrate the instrument after each reading. If the unknown gives a reading lower than beaker 3, dilute it in a known manner and remeasure (example 2 mL to 5 mL). You will need to account for this dilution.

You will take readings in the % transmittance mode. You can use your calculator to convert to absorbance.

WASTE DISPOSAL:

All solutions may be flushed down the drain with plenty of tap water.

Experiment 4: Introduction to Spectroscopy

Data Sheet 1

Name_____ Lab Section_____

Lab Partners:_____

Dye color#1: _____ Initial Concentration _____

AnalyticalWavelength (λ max) _____

Soln #	Dye (mL)	H_2O (mL)	Concentration (ppm)	%T	Absorbance
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Dye color#2: _____ Initial Concentration _____

AnalyticalWavelength (λ max) _____

Soln #	Dye (mL)	H_2O (mL)	Concentration (ppm)	%T	Absorbance
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Experiment 4: Introduction to Spectroscopy

Data Sheet 2

Name_____ Lab Section_____

Unknown #: _____

Dye color#1: _____ AnalyticalWavelength (λ max) _____

Equation of Line for Dye Color #1 _____

Unknown %Transmittance: _____ Unknown Absorbance: _____

Concentration of Dye Color #1 in the unknown solution: _____

Dye color#1: _____ AnalyticalWavelength (λ max) _____

Equation of Line for Dye Color #2 _____

Unknown %Transmittance: _____ Unknown Absorbance: _____

Concentration of Dye Color #1 in the unknown solution: _____

Experiment 3: Introduction to Spectroscopy

Postlab Exercise

Name_____ Lab Section_____

1. A UV/Vis spectrometer is used to measure the Absorbance of a solution of hemoglobin bound to oxygen at 590 nm. The molar absorptivity was determined to be $15.5 \times 10^3\ M^{-1}cm^{-1}$. The cuvette used had a 1.00 cm sample pathlength. The measured absorbance was _____. What was the concentration of hemoglobin in the solution?

2. What would the absorbance of the solution in question 1 be if the pathlength were 2.00cm?