

Experiment

CRYSTAL VIOLET COLORIMETRY: A BEER'S LAW INVESTIGATION

The CCLI Initiative

Computers in Chemistry Laboratory Instruction

LEARNING OBJECTIVES

The objectives of this exercise are to . . .

- illustrate the basic principles of colorimetry.
- demonstrate the components of a colorimeter and how a colorimeter is interfaced to a computer.
- discover the Beer's Law relationship and apply it to the analysis of an unknown solution.

BACKGROUND

Colorimetry is an instrumental method based on the measurement of light absorption by colored solutions and is widely used for performing chemical analyses. The *MicroLAB* colorimeter utilizes 10 Light Emitting Diodes (LEDs) ranging from 400 to 644 nanometers, spaced about 35 nanometers apart. They are scanned sequentially and the transmittance or absorbance data is presented as 10 colored bars in a bar graph as shown in Figure 1.

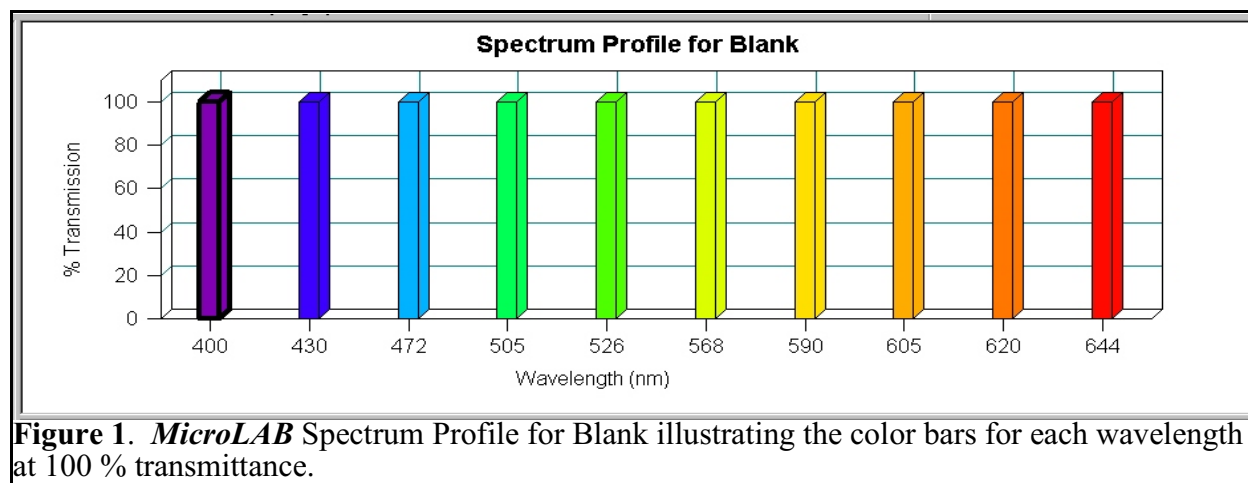
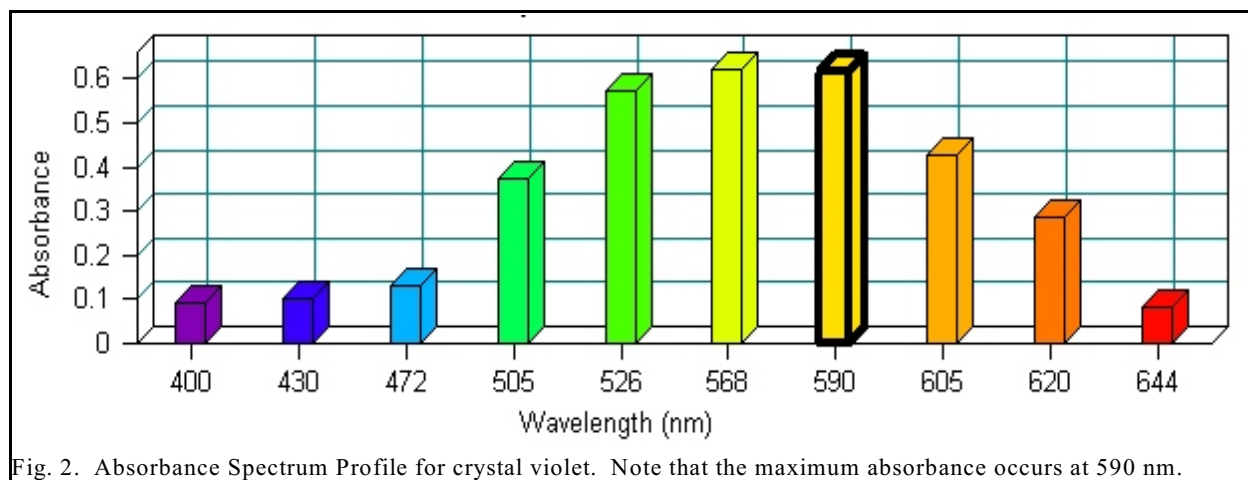


Figure 1. *MicroLAB* Spectrum Profile for Blank illustrating the color bars for each wavelength at 100 % transmittance.

These components are arranged such that light from each LED pass through the crystal violet solution and falls on a photocell. The photocell circuit produces a current in microamps (I) which is proportional to the light intensity striking the photocell surface and is converted into **Transmittance** and **Absorbance** by the *MicroLAB* software. These components are all housed in the colorimeter chamber of the *MicroLAB* interface.

Crystal violet (also called methyl violet) is an organic dye. In crystal violet solutions where the molarity of H^+ is greater than 0.1, the solution color is yellow. If H^+ molarity less than 0.1, the solution color is purple. Our studies will involve purple solutions of the dye.

In this experiment you will prepare five crystal violet solutions of known concentrations varying from $2.00 \times 10^{-6} M$ to $10.0 \times 10^{-6} M$. The Transmittance for each of these *standard* solutions, for a water blank, and for a crystal violet solution of unknown concentration will be measured. The **Transmittance** should decrease with increasing solution concentration while the **Absorbance** should increase with increasing solution concentration. The Spectrum Profile for the absorbance of a CV solution is shown in Figure 2. Note that the maximum absorbance is at the 590 nm wavelength.



One objective of this experiment is to find a mathematical function of **Transmittance** that is directly proportional to sample concentration. Such a function does exist and forms the basis for what is known as **Beer's Law**. A graph of this function vs. concentration, which is linear and intersects the origin, is called a Beer's Law plot. To explore this **concentration/Transmittance** relationship, you will first **Hand Enter** some "ideal" simulated data into the spreadsheet program. You will compute and graph several current functions (the square, reciprocal, and logarithm) until a function is discovered that has a direct proportionality to concentration. This discovered function is then used for creating a Beer's Law plot from your actual data. Finally, the concentration of the unknown crystal violet solution is obtained directly from this plot.

SAFETY PRECAUTIONS

Crystal violet solutions may cause skin and eye irritation. Safety goggles must be worn at all times. As usual, wash hands with soap and water before leaving the laboratory.

BEFORE PERFORMING THIS EXPERIMENT . . .

...you will need the *MicroLAB Hand Enter* and *Colorimetry Experiment* programs

EXPERIMENTAL PROCEDURE

Simulated concentration/current data treatment

1. Open the *MicroLAB* in the **Hand Enter** mode and enter the following "ideal" simulated data into the *MicroLAB* spreadsheet program. Use Column A for the concentration values and label as Molarity. Use Column B for the current values and label as Current (I).

<i>Concentration (M)</i>	<i>Current (I)</i>	<i>Concentration (M)</i>	<i>Current (I)</i>
0.00 x 10 ⁻⁶	600.0	6.00 x 10 ⁻⁶	300.6
2.00 x 10 ⁻⁶	476.4	8.00 x 10 ⁻⁶	238.8
4.00 x 10 ⁻⁶	378.6	10.0 x 10 ⁻⁶	189.6

2. After entering the data, "click and drag" transmittance from the **Spreadsheet** to the *y-axis* and concentration to the *x-axis*. Click on **Analysis**, then **Add a Curve Fit**, select the variables, then, here and elsewhere, select **Linear Curve fit** as the type of plot desired, and the spreadsheet program will fit the data with the best possible straight line. Note in this case, the data points would be better fit by a curved line, and hence, the correlation (Corr.) is poor. An acceptable Correlation value for a linear plot would be at least 0.999. Print this graph for your report.
3. Using the **Add Formula** function, in turn, try graphing I², 1/I, and Log(I) (*y-axis*) vs. concentration. When you discover the linear plot, leave the graph on the screen and wait until all groups arrive at the same point. Your instructor will then discuss the modifications needed to give a straight line with a *positive* slope which also passes through the *origin*.

Development of Beer's Law

Use the space provided below to make notes on the development of Beer's Law as provided by your instructor.

EXPERIMENTAL PROCEDURES

Solution preparation

A stock 10.0 x 10⁻⁶ M crystal violet (CV) solution is provided. Perform dilution calculations to complete the following table (see your text if needed) and have your work checked for correctness by your lab instructor.

Dilution Table for Standards

	#1	#2	#3	#4	#5
Desired CV Molarity	2.00 x 10 ⁻⁶	4.00 x 10 ⁻⁶	6.00 x 10 ⁻⁶	8.00 x 10 ⁻⁶	10.0 x 10 ⁻⁶
mL CV					
mL H₂O					
Total mL	15.00	15.00	15.00	15.00	15.00

Use the burets provided to transfer the volumes calculated in the above table into a series of small, dry bottles. Use great care in your buret measurements; careless work will be rewarded with unacceptable Beer's Law plots and the necessity of repeating the experiment. When all five solutions have been prepared, cap

them tightly and mix by gently inverting the vials several times to avoid bubbles. Be sure to label each solution according to the above numbering.

Measurements

1. Select **Colorimeter Experiment** from the **Main *MicroLAB* Menu** to carry out the experiment. Your data will come from the ***MicroLAB* Interface**.
2. Your first sample should be the water blank (0.00 *M*). Place a vertical mark on the vial so that you can insert the vial in the colorimeter the same way each time. Fill the colorimeter vial about three-fourths full, wipe the outside faces with a KimWipe, insert the vial into the colorimeter with the mark facing forward and place the light cover over the vial. The program will automatically shift to **Read Knowns** after the Blank read.
3. For each sample of CV, rinse the vial three times with small amounts of the solution, then fill the vial three-fourths full, insert it into the colorimeter, place the cap over the vial and click the **ADD** button.
4. The program will direct you to enter the concentration. It will then display the transmittance for that sample. Follow this procedure for each of the standard solutions in order of increasing concentration. Be sure to wipe the vial with a KimWipe each time.
5. For your unknown solution, click on **Read Unknowns** and measure the transmittance in the same manner as was done for the standards.

DATA ANALYSIS

1. Obtain printouts of:
 - a. each of your **Hand Entered** data graphs, as modified by the Add Formula for each mathematical transformation, and calculated absorbance values and
 - b. your Beer's Law plot with grid.
2. Use your Beer's Law plot regression equation to calculate the concentration of the unknown, then compare it against the value given on the screen. You must show your calculations for credit.